ANALYTICAL METHOD DEVELOPMENT FOR SODIUM BENZOATE AND ITS APPLICATION TO SOFT DRINKS AND FRUIT JUICES

A Dissertation submitted to

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY,

CHENNAI – 600 032

In partial fulfilment of the requirements for the award of the Degree of

MASTER OF PHARMACY
IN
BRANCH–V- PHARMACEUTICAL ANALYSIS

Submitted by

SANGEETHA.S
REGISTRATION No.261430105

Under the guidance of

Dr. M.GANDHIMATHI, M.Pharm., Ph.D.,
Department of Pharmaceutical Analysis

COLLEGE OF PHARMACY
SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES
Coimbatore - 641 044.

OCTOBER 2016

CERTIFICATE

This is to certify that the dissertation entitled **Analytical Method Development for Sodium Benzoate and its Application to Soft Drinks and Fruit Juices** being submitted to The Tamil Nadu Dr.M.G.R Medical University, Chennai was carried out by **Sangeetha.S** in the **Department of Pharmaceutical Analysis,** College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under the supervision and guidance of **Dr.M.Gandhimathi, M.Pharm., Ph.D.,** Associate Professor, Department of Pharmaceutical Analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore.

Dr. T. K. RAVI, M. Pharm., Ph.D., FAGE.,
Principal & HOD,
Department of Pharmaceutical Analysis,
College of Pharmacy,
SRIPMS,
Coimbatore-641 044.

Place: Coimbatore

Date:

CERTIFICATE

This is to certify that the dissertation entitled **Analytical Method Development for Sodium Benzoate and its Application to Soft Drinks and Fruit Juices** being submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai was carried out by **Sangeetha.S** in the Department of Pharmaceutical Analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore under my direct supervision and guidance to my fullest satisfaction.

Dr. M. GANDHIMATHI, M.Pharm., Ph.D.,
Associate Professor,
Department of Pharmaceutical Analysis,
College of Pharmacy,
SRIPMS,
Coimbatore-641 044

Place: Coimbatore

Date:

CERTIFICATE

This is to certify that the dissertation entitled **Analytical Method Development for Sodium Benzoate and its Application to Soft Drinks and Fruit Juices** being submitted to The Tamil Nadu Dr.M.G.R Medical University, Chennai was carried out by **Sangeetha.S** in the **Department of Pharmaceutical Analysis,** College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, under the supervision and guidance of **Dr.M.Gandhimathi, M.Pharm., Ph.D.,** Associate Professor, Department of Pharmaceutical Analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore.

Dr. T. K. RAVI, M. Pharm., Ph.D., FAGE.,
Principal & HOD,
Department of Pharmaceutical Analysis,
College of Pharmacy,
SRIPMS,
Coimbatore-641 044.

Place: Coimbatore

Date:

CERTIFICATE

This is to certify that the dissertation entitled **Analytical Method Development for Sodium Benzoate and its Application to Soft Drinks and Fruit Juices** being submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai was carried out by **Sangeetha.S** in the Department of Pharmaceutical Analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore under my direct supervision and guidance to my fullest satisfaction.

Dr. M. GANDHIMATHI, M.Pharm., Ph.D.,
Associate Professor,
Department of Pharmaceutical Analysis,
College of Pharmacy,
SRIPMS,
Coimbatore-641 044

Place: Coimbatore

Date:

ACKNOWLEDGEMENT

I would like to devote my sincere gratitude to my guide ***Dr. M. Gandhimathi, M.Pharm., Ph.D.,*** *Associate Professor, Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore for her remarkable guidance and valuable suggestion during the tenure of my work. I wish to convey my deep sense to her for all the guidance she provided me over the time of my academic years. There is no doubt that without her efforts the task would not be achieved. It is my great privilege to have such dedicated guide like her that provides dynamic encouragement to me.*

It is my pleasure to express my sedulous gratitude to our Principal ***Dr. T. K.Ravi M.Pharm., Ph.D., FAGE,*** *College of Pharmacy, SRIPMS, Coimbatore for giving us an opportunity to do this project work and for providing all necessary facilities for it.*

I extend my gratitude and respectful regards to our Managing Trustee, ***Thiru.K.Vijayakumhar*** *and the Management of SRIPMS for providing complete facilities required for the work.*

My solemn thanks to my dear teachers, ***Dr. R. Jayapakasam, M.Pharm, Ph.D., Dr. A. Suganthi, M.Pharm., Ph.D., Dr. Susheel John Varghese, M.Pharm., Ph.D.,*** *and* ***Mrs. Sangeetha Sudhakaran, M.Pharm.,*** *Department of Pharmaceutical Analysis, College of Pharmacy, SRIPMS, Coimbatore for their timely help and guidance during the course of the work.*

My special thanks to ***Mrs. B. Chitra, M.Pharm., (Ph.D).,*** *Department of Pharmacy Practice for her valuable suggestion during my course.*

A special thanks to ***Mrs. Kalaivani*** *and* ***Mrs. Dhanalakshmi*** *for unstinted support at the time of need.*

My heartfelt thanks to my dear friends ***Arthi, Dhivya, Rajendran, Mohanraj, Mahesh, Prasanth, Ramseela, Naveen, Sathesh, Aravind, Sumithra, Gobi, Arya, Geena, Guna*** *who have helped me during my project work.*

Above all I dedicate myself before the constant love and encouragement given to me by my beloved Parents ***Mr.Sathyanathan. A*** *and* ***Mrs.P.K.Sushama*** *who have been the backbone of all my achievements.*

*Above all, I bow with reverence before the gracious presence and boundless blessings of "****The Almighty****" who is the source of all wisdom and knowledge for the successful completion of this thesis work.*

CONTENTS

LIST OF ABBREVIATIONS

gm	-	Gram
HPLC	-	High Performance Liquid Chromatography
ICH	-	International conference on harmonization
LOD	-	Limit of detection
LOQ	-	Limit of quantification
M	-	Molar
mg	-	Milligram
min	-	Minute
ml	-	Millilitre
mM	-	Millimolar
RP-HPLC	-	Reverse phase high performance liquid chromatography
R_s	-	Resolution
RSD	-	Relative standard deviation
R_t	-	Retention time
T_f	-	Tailing factor
λmax	-	Wavelength of maximum absorbance
µg	-	Microgram
µL	-	Microlitre

INTRODUCTION[1-15]

Food analysis is the discipline dealing with the development, application and study of analytical procedures for characterizing the properties of foods and their constituents. These analytical procedures are used to provide information about a wide variety of different characteristics of foods, including their composition, structure, physicochemical properties and sensory attributes. This information is critical to our rational understanding of the factors that determine the properties of foods, as well as to our ability to economically produce foods that are consistently safe, nutritious and desirable and for consumers to make informed choices about their diet.

Foods are analyzed by scientists working in all of the major sectors of the food industry including food manufacturers, ingredient suppliers, analytical service laboratories, government laboratories, and University research laboratories.

GOVERNMENT REGULATIONS:

Government regulations and recommendations are designed to maintain the general quality of the food supply, to ensure the food industry provides consumers with foods that are wholesome and safe, to inform consumers about the nutritional composition of foods so that they can make knowledgeable choices about their diet, to enable fair competition amongst food companies, and to eliminate economic fraud. There are a number of Government Departments Responsible for regulating the composition and quality of foods, including the Food and Drug Administration (FDA), the United States Department of Agriculture (USDA), the National Marine Fisheries Service (NMFS) and the Environmental Protection Agency (EPA). Each of these government agencies is responsible for regulating particular sectors of the food industry and publishes documents that contain detailed information about the regulations and recommendations pertaining to the foods produced within those sectors.

FOOD SAFETY

One of the most important reasons for analyzing foods from both the consumers and the manufacturer's standpoint is to ensure that they are safe. It would be economically disastrous, as well as being rather unpleasant to consumers, if a food manufacturer sold a product that was harmful or toxic. A food may be considered to be unsafe because it contains harmful microorganisms (*e.g.*, Listeria, Salmonella), toxic chemicals (*e.g.*, pesticides, herbicides) or extraneous matter (*e.g.*, glass, wood, metal, insect matter). It is therefore important that food manufacturers do everything they can to ensure that these harmful substances are not present, or that they are effectively eliminated before the food is consumed. This can be achieved by following good manufacturing practice regulations specified by the government for specific food products and by having analytical techniques that are capable of detecting harmful substances. In many situations it is important to use analytical techniques that have a high sensitivity, *i.e.*, that can reliably detect low levels of harmful material. Food manufacturers and government laboratories routinely analyze food products to ensure that they do not contain harmful substances and that the food production facility is operating correctly.

RESEARCH AND DEVELOPMENT

In recent years, there have been significant changes in the preferences of consumers for foods that are healthier, higher quality, lower cost and more exotic. Individual food manufacturers must respond rapidly to these changes in order to remain competitive within the food industry. To meet these demands food manufacturers often employ a number of scientists whose primary objective is to carry out research that will lead to the development of new products, the improvement of existing products and the reduction of manufacturing costs.

PRESERVATIVES FOR FOOD

Preservatives are commonly added to many food products, such as soda, fruit juice, soy sauce, jams and jellies, and other condiments, to inhibit decay. Since the early 1900s, benzoate has been widely used worldwide as a preservative due to its antimicrobial properties combined with its low toxicity and taste. Benzoate is most effective in an acidic environment (pH $\leq$4.5) and is not recommended for use at higher pH. Benzoic acid is an effective antimicrobial agent for the purpose of preservation. However, sodium benzoate is more effective and preferred because it is approximately 200 times more soluble than benzoic acid.

The soft drink industry is the largest user of benzoate as a preservative due to the amount of high fructose corn syrup in many carbonated beverages. Soft drinks account for the largest human consumption of benzoate in the USA, Australia, New Zealand, France, and the United Kingdom. Although soft drinks do not normally spoil due to their acidity and carbonation, preservatives are required to prevent changes during long-term storage.

The Food and Drug Administration (FDA) regulates the uses of benzoate as a preservative in the USA. The FDA lists benzoate as a substance that is generally recognized as safe (GRAS) with a maximum permitted concentration of 0.1% in accordance with good manufacturing or feeding practices. Similarly, benzoate is regulated in Europe by the European Union Legislation (Directive 95/2/EC) with a limit of 0.015% in soft drinks and up to 0.2% in other food products. If higher concentrations of benzoate are used (~0.1%), then alterations in taste may occur in soft drinks.On the other hand, concentrations less than 0.010% will have little inhibitory effect. Therefore, a reliable testing method is required to assure that the concentration of benzoate is within product and regulatory specifications.

Methods used to determine benzoic acid or its corresponding salt in foods, beverages, and other matrices include titrimetry, ion-selective electrodes, gas chromatography (GC), thin-layer chromatography, and high-performance liquid chromatography (HPLC). Many of these methods have significant disadvantages and are therefore not preferred for use in a quality control environment if a large number of samples are to be analyzed. For example, the GC method proposed by the Association of Official Analytical Chemists for the determination of benzoic acid and sorbic acid requires solvent extractions and derivatization techniques. This process involves complex procedures and is exceptionally time-consuming. From the previously listed techniques, HPLC (including reversed phase, ion exchange, and ion exclusion) is used most often for the determination of benzoic acid. With this technique, many samples can be simply diluted and injected directly into the chromatography system without any complex sample preparation. In this application note, we describe a simple ion chromatography method for the direct determination of benzoate in liquid food products.

CLASS I PRESERVATIVES

- Common salt
- Sugar
- Dextrose
- Glucose
- Spices
- Vinegar or acetic acid
- Honey
- Edible vegetable oils

CLASS II PRESERVATIVES

- Benzoic acid including salts thereof
- Sulphurous acid including salts thereof
- Nitrates or nitrites of sodium or potassium in respect of foods
- Sorbic acids and its sodium, potassium, and calcium salts
- Methyl or propyl para hydroxy benzoates Sodium Diacetate
- Propionates of calcium or sodium, lactic acid and its sodium, potassium and calcium salts and calcium phosphate

HARMFUL EFFECT OF PRESERVATIVES:

There are certain harmful effects of using chemicals for preservation such as

1. Sulphites:

Sulphites are common preservatives used in various fruit juices, may have side effects in form of headaches, palpitations, allergies, and even cancer.

2. Nitrates and Nitrites:

These additives are used as curing agents in meat products. It gets converted into nitrous acid when consumed and is suspected of causing stomach cancer.

3. Benzoates:

Benzoates are used in foods as antimicrobial preservatives, and have been suspected to cause allergies, asthma and skin rashes.

4. Sorbates:

Sorbates / sorbic acid are added to foods as antimicrobial preservatives. Reactions to sorbates are rare, but have included reports of urticaria and contact dermatitis.

E NUMBER:

E Numbers are number codes for food labels. This is international numbering system (INS)

E stands for Europe

E100-E199 - colors

E200-299 - preservatives

E 300-E399 - antioxidants, acidity regulators.

Sl.No	E Number	Preservative
1	E200-209	Sorbates
2	E210-219	Benzoates
3	E220-E229	Sulphites
4	E230-E239	Phenols and formats
5	E240-E259	Nitrates
6	E260-E269	Acetates
7	E270-E279	Lactates
8	E280-E289	Propionates
9	E290-E299	Others

CLASS II: preservatives are mostly used in health drinks, pharmaceutical preparations and food stuffs.

E210 benzoic acid

E211 sodium benzoate

E216 propyl parabene

E218 potassium metabisulphite

NEED OF PRESERVATIVES:

The considerable time between the production and the consumption of food today makes use of preservatives necessary in order to prevent spoilage and undesirable alterations in color, flavour, or nutrients. Degradation pathways for benzoic acid (produced in the body from the sodium salt) have been studied in detail and have shown the harmlessness of this substance: 75-80% is excreted within 6 hours, and the total dose leaves the body within about 10 hours. It does not cause cancer.

The limit of sodium benzoate in foods is not because of its toxicity, but at levels higher than 0.1% will have an acceptable aftertaste. Foods containing this preservative are much healthier than non-preservative foods since harmful microorganism growth is inhibited, food oxidation is prevented, and food nutrients are preserved.

Chronic toxicities were examined in rats fed diets containing up to a total of 1%. After 4 generations there cosmetic were no changes in normal patterns of growth, reproduction, lactation and no morphological abnormalities of organs. Acute toxicity studies, where one large dose of sodium benzoate is given to animals, showed no lethal effects until 2 grams per kg body weight was administered. One could not eat enough foods containing sodium benzoate to even get 0.002% of this amount.

Sodium benzoate

Sodium benzoate (E211), also called benzoate of soda, has the chemical formula $NaC_6H_5CO_2$. It is the sodium salt of benzoic acid and exists in this form when dissolved in water. It can be produced by reacting sodium hydroxide with benzoic acid.

USES:

Sodium benzoate is a preservative. It is bacteriostatic and fungistatic under acidic conditions. It is used prevalently in acidic (pH less than 3.6) in foods such as salad dressings (vinegar), and condiments. It is also found in alcohol based mouthwash and silver polish. It can also be found in cough syrups.

It is also used in fireworks as a fuel in whistle mix, a powder which imparts a whistling noise when compressed into a tube and ignited. It is found naturally in cranberries, prunes, greengage plums, cinnamon, ripe cloves, and apples. Concentration as a preservative is limited by the FDA in the US to 0.1% by weight though natural cranberries and other fruit varieties contain levels of sodium benzoate approaching this limit. Clague. et.al, (1934).

Cats have a significantly lower tolerance against benzoic acid and its salts than rats and mice. Sodium benzoate is, however, allowed as an animal food additive at up to 0.1%, according to AFCO's official publication. AFCO, (2004).

MECHANISM OF FOOD PRESERVATION:

The mechanism starts with the absorption of benzoic acid into the cell. If the intercellular p^H changes to 5 or lower, the anaerobic fermentation of glucose through phosphofructokinase is decreased by 95%.

SAFETY AND HEALTH:

In combination with ascorbic acid (vitamin C, E300), sodium benzoate and potassium sorbate may form benzene, a known carcinogen. FDA,(2006). Heat, light and shelf life can affect the rate at which benzene is formed.

Professor peter piper of the university of Sheffield claimed that sodium benzoate by itself can damage and inactivate vital parts of DNA in a cells mitochondria. Mitochondria consume oxygen to generate ATP, the body's energy currency. If they are damaged due to disease, the cell malfunctions may enter apoptosis. There are many illnesses now tied to DNA damage, including Parkinson's and other neurodegenerative diseases, but above all, aging process in general.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High performance liquid chromatography is a major tool that can be applied in all levels of drug discovery, development and production. It can also be used for qualitative and quantitative analysis. In this method mobile phase is allowed to move through a packed column. The sample that has to be analysed is injected along with the mobile phase to the column. The sample will be separated based on the affinity towards the stationary phase in the column.

Types of HPLC

- Normal phase HPLC
- Reversed phase HPLC
- Ion-exchange HPLC
- Size-exclusion HPLC

Most of the pharmaceutical products are analysed by RP-HPLC.

HPLC system

HPLC system consists of following main parts

- Solvent reservoirs- These to keep the HPLC solvents which are used as mobile phase. It could be equipped with online degassing system.
- Pump- These are the components that allows the continuous and constant flow of the mobile phase through the system
- Injector- This allows to inject the sample or analyte mixture that we have to analyse into the stream of mobile phase which enters into the column. Modern injectors are auto samplers which are programmable.
- Column- It is the heart of HPLC system which separates the analytes in the mixture which we are injecting. This is the place where mobile phase is in contact with stationary phase.
- Detector- This is a device used for the registration of the specific properties of the column effluent.

Steps involved in HPLC method development are:

- Understand the physicochemical properties of drug molecule.
- Set up HPLC conditions.
- Preparation of sample solution for method development.
- Method optimization.
- Validation of method.

Understand the physicochemical properties of drug molecule

Physicochemical properties of a drug molecule play an important role in method development. For Method development the physical properties like solubility, polarity, pKa and pH of the drug molecule are studied.

Set up chromatographic condition

This include selection of buffer, detector, column, mobile phase, mode of separation etc.

Method optimization

The experimental conditions should be optimized to get desired separations and sensitivity after getting appropriate separations. This can be achieved by examination on parameters including pH, mobile phase components and ratio, gradient, flow rate, temperature, sample amounts, Injection volume and diluents solvent type.

Detection of compounds

The detectors used in a HPLC systems are solute property detectors (UV, fluori, PDA) and bulk property detector (refractive index). Drugs which have chromophores can be easily detected by solute property detectors but if chromophores are absent several steps has to be done for detecting using a UV or PDA detector.

Validation of method

All analytical methods that are intended to be used for analyzing any samples will need to be validated. The validation of analytical methods is done as per ICH guidelines.

Parameters Used in Chromatographic Characterization

Column efficiency (N)

The efficiency of a column is expressed by the number (N) of theoretical plates in the column or by the height equivalent of a theoretical plate (HETP). The larger the number of theoretical plates or the smaller the HETP, the more efficient the column is for separation.

$N = 16(t_R/w)\ 2$ or $3500L\ (cm)/\ dp\ (\mu m)$

Where, L = Column length

T_R = Band retention time

dp = Particle diameter

Retention

The retention of a drug with a given packing material and eluent can be expressed as retention time or retention volume, but both of this are dependent on flow rate, column length and column diameter. The retention is best described as a column capacity ratio (k), which is independent of these factors. The column capacity ratio of a compound (A) is defined as

$$k_A = \frac{V_A - V_o}{V_o} = \frac{t_A - t_o}{t_o}$$

Where V_A = Elution volume of A

V_0 = Elution volume of a non-retained compound (void volume**)**

Resolution

The distance between any two adjacent peaks in a multi peak chromatogram is referred to as Resolution 'Rs' and is calculated as

$$R = \frac{2(t_2 - t_1)}{W_1 + W_2}$$

Where t_1 and t_2 are the retention time for the latest and the earliest eluting peak and W_1 and W_2 are the peak width at base line.

$R \geq 1$ = Components completely separated

$R \leq 1$ = Component overlap

Capacity Factor (k')

The retention of the analyte is expressed as the number of void volumes of the system, needed for the peak to elute is called the capacity factor. The expression for k is

$$k' = \frac{t_r - t_0}{t_0}$$

Where t_r= retention time

t_o = void volume

Theoretical Plate (N)

The number of theoretical plates generated on a column is a measure of its performance. The definition of N is

$$N = 5.54\left(\frac{t_r}{t_{W1/2}}\right)$$

Where tr – retention time

$t_{w\,½}$ -is the peak width at half height

'N' may also be calculated from the width along the baseline of the peak. This is accomplished by extending tangents from the two peak inflection points through the baseline.

Separation Factor (α)

This parameter is used to quantify the separation between adjacent peaks. Ideally, the peaks should not overlap, that is they should be baseline resolved. This condition is met for peaks of similar size when $\alpha > 1.15$.

The separation factor is calculated as follows

$$\alpha = \frac{K_2'}{K_1'}$$

Where, The subscripts refer to the order of elution. α is always ≥ 1

Asymmetry

The asymmetry is a tool for rapidly determining how much if any, of an eluting peak profile in shape from a standard distribution. The subscript 'X' refers to the percentage of peak height at which the asymmetry is determined.

Eg A_{10} (determined at 10% peak height)

Peak asymmetry is given as;

$A_x = b/a$

Where,

b = distance between the perpendicular join the baseline to peak maximum and the latest eluting portion of the curve

a = the distance between the perpendicular join the baseline to the peak maximum and the earliest eluting portion of the curve.

Tailing factor

The tailing factor is an estimation of peak tailing. It is described as the interval from the front slope of the peak to the back slope cleaved by twice the distance from the center line of the peak to the front slope, with all computation made at 5% of the maximum peak height. The USP suggests that Tailing Factor should be in the range of 0.5 up to 2 to assure a precise and accurate quantitative measurement.

VALIDATION OF ANALYTICAL METHOD

Analytical Parameters to be validated

1. Analytical Procedure

The analytical procedure refers to the way of conducting the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus generation of the calibration curve, use of the formulae for the calculation, etc.

2. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

This definition has the following implications:

Identification:

To ensure the identity of an analyte.

Purity Tests:

To ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Assay (content or potency):

To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

3. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

This is sometimes termed trueness.

4. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

a. Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

b. Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

c. Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

5. Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

6. Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyle in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

7. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

8. Robustness

The robustness of a method is its ability to remain unaffected by small changes in parameters such as percentage organic concentration and pH of mobile phase, concentration of buffer, temperature, flow rate and injection volume.

REVIEW OF LITERATURE[16-25]

An extensive literature survey revealed that there are few methods reported for the analysis of sodium benzoate and they are described below

- S. Antakli et.al reported a method for the determination of sodium benzoate and potassium sorbate preservatives in foodstuffs using high performance liquid chromatography. Separation and determination of sodium benzoate and potassium sorbate were performed on a 5µm Purospher® STAR RP-18column (25 cm × 4.6 mm), using caffeine as an internal standard (0.04mg/mL), with UV detection at 235 nm. Mobile phase was (acetate buffer and methanol) with percentage (25:75), at a flow rate of1.2 mL/ min.

- Khosrokhavar Ret.al., reported a method to determine the preservatives (sodium benzoate and potassium sorbate) in soft drinks and herbal extracts using high-Performance liquid chromatography (HPLC). The separation of Sodium Benzoate and Potassium Sorbate were performed on the C_{18}- column and acetonitrile –ammonium acetate buffer in the ratio 40:60 were used as mobile phase. The detector wavelength was set at 254 nm.

- Ahmet kucukcetinetet.al., reported a method to estimate sodium benzoate, potassium sorbate, nitrate and nitrite in some commercial diary products. The percentage of sodium benzoate present in different dairy products were 74%, 85% and 80% and presence of potassium sorbate was within the legal limits. The presence of nitrites have been found were low.

- KJ. Tidke et al.; reported on estimation of preservative from local market beverages of Amravati (M.S.) by hplc technique. The HPLC analysis was performed by isocratic elution with mobile phase acetonitrile and ammonium acetate buffer 4.2 pH in 40:60 respectively. UV detection was carried out at 228nm by using phenomenex C18 column (250 × 4.6 mm, 5µ) at ambient temperature and flow rate of 1ml/min.

- Vilas Khade et.al.; developed a high performance liquid chromatography method for analysis of sodium benzoate. The chromatographic separation was obtained by isocratic elution of mixture of buffer and methanol (60:40v/v) on C_{18} column with UV detection at 254 nm.

- Zahra Esfandiari et al., reported a method to determine sodium benzoate and potassium sorbate and natamycin content in Iranian yoghurt drink (doogh).39 Doogh samples were analysed through RP-HPLC. All the samples contain sodium benzoate concentration range from 0.94 to 9.77 mg/ml. The percentage of natamycin detected was 10.25% of the sample and potassium sorbate was not detected.

- Pylypiw HW JR and Grether MT performed a rapid HPLC method for the analysis of sodium benzoate and potassium sorbate by using C_{18} Column. The detection was done at 225nm and 255nm for sodium benzoate and potassium sorbate respectively. The method could detect 0.0010% of preservative in juice matrix.

- Goksel Altiokka et al., reported a validated RP-HPLC method for the analysis of the food additive, sodium benzoate, in soft drinks and jams. Chromatographic separation was achieved using a C_{18} reversed phase column and methanol: water (70:30, v/v) adjusted to pH 3.45 with glacial acetic acid as mobile phase, 0.45 mL min^{-1} flow rate, and UV detection at 245 nm.

- Bahruddin Saada et al. developed a method to determine the preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in foodstuffs using high-performance liquid chromatography. The separations were effected by using an initial mobile phase of methanol–acetate buffer (pH 4.4) (35:65) to elute Benzoic acid, Sorbic acid and Methyl paraben and changing the mobile phase composition to methanol–acetate buffer (pH4.4) (50:50). The detector wavelength was set at 254 nm. Under these conditions, separation of the four components was achieved in less than 23 min.

- Burge Asci et al. reported on development and validation of HPLC method for the simultaneous determination of five food additives and caffeine in soft drinks. The experimental variables chosen were pH (6.0–7.0), flow rate (1.0–1.4 mL/min) and mobile phase ratio (85–95% acetate buffer). Resolution values of all peak pairs were used as a response. Stationary phase was Inertsil Octa Decyl Silane- (ODS-) 3V reverse phase column (250 × 4.6 mm, 5 μm). The detection was performed at 230 nm.

AIM AND PLAN OF WORK

The present work aims to develop a newer analytical method that is simple, accurate, precise and economic for the determination of sodium benzoate and its application to selected soft drinks and fruit juices.

The plan includes:

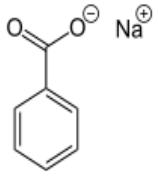
1. Development and validation of RP-HPLC method for sodium benzoate.
2. Application of HPLC method for the quantification and estimation of sodium benzoate from selected soft drinks and fruit juices.

PROFILE OF SODIUM BENZOATE

PROFILE OF SODIUM BENZOATE

Name : SODIUM BENZOATE

Structure :



Molecular formula : C_6H_5COONa

Molecular weight : 144.103

Category : Preservative

Melting point : 410°c

Wavelength : 232 nm

Description : White crystalline powder

Solubility : Soluble in methanol

Storage : Store at room temperature

Functions : Bacteriostatic and fungistatic under food as preservatives acidic condition

Mechanisms of action	:	Mechanism starts with absorption of Food preservative benzoic acid into the cell. If the Intercellular pH falls to 5 or lower, the anerobic fermentation of glucose through phosphofructokinase decrease sharply which inhibit the growth and survival of microorganisms that cause food spoilage.
Excretion	:	Transported to liver where it is filtered and expelled in urine, Safety limit used in food Concentration of preservative is limited by FDA to 0.1% by weight.
Adverse effect	:	It rarely causes side effect when consumed in large doses. In certain individuals nausea, vomiting may occur, can irritate existing stomach ulcer, causes mild hyperventilation, possibly leading to dizziness.

PROFILE OF SOFT DRINKS AND JUICES

Soft drinks:

Product-1

Product Name	:	Miranda
Net content	:	600 ml
Batch No.	:	BN-947B30D16
Mfg. Date	:	30-4-2016
Preservative	:	Sodium Benzoate (E211)

Product-2

Product Name	:	7UP
Net content	:	600 ml
Batch No.	:	AN BN.7116 G 27 .E16
Mfg. Date	:	27.05.2016
Preservative	:	Sodium Benzoate (E211)

Product-3

Product Name	:	Revive Hydrotonic Drink
Net content	:	330 ml
Batch No.	:	BN-947B29016
Mfg. Date	:	26.04.2016
Preservative	:	Sodium Benzoate (E211)

FRUIT JUICES

Product-4

Product Name	:	Frooti
Net content	:	600 ml
Batch No.	:	BN FBP 16121
Mfg. Date	:	30.04.2016
Preservative	:	Sodium Benzoate (E211)

Product-5

Product Name	:	Maa
Net content	:	600 ml
Batch No.	:	BN A141L1
Mfg. Date	:	15.04.2016
Preservative	:	Sodium Benzoate (E211)

MATERIALS AND INSTRUMENTS

CHEMICALS AND SOLVENTS USED

- Sodium benzoate
- Water HPLC grade
- Methanol HPLC grade, AR grade
- Orthophosphoric acid–AR grade
- Ammonium acetate – AR grade
- Diethyl ether – AR grade

All the above chemicals and solvents were supplied by S.D. Fine chemicals Ltd., India, Sigma-aldrich Chemicals Pvt. Ltd., Maharashtra, India and Ranbaxy chemicals Ltd., New Delhi, India.

The Soft drinks mirinda,7up, revive hydrotonic drink and fruit juices frooti, maa were purchased from local market.

MATERIALS USED

- Lichro CART, C_{18} column (250mm×4.0mm, 5µm**)** column from Merck Pvt. Ltd., Mumbai.

INSTRUMENTS USED

1. Shimadzu digital electronics balance
2. Elico Pvt. Limited, India, pH meter
3. Jasco V-600 UV/ Vis- spectrophotometer
4. Shimadzu HPLC system with SPD-M10 A VP system PDA with 20µl fixed volume manual injector and LC-MS solution software.

EXPERIMENTAL SECTION

DEVELOPMENT OF RP-HPLC METHOD FOR SODIUM BENZOATE

Selection of solvent

Sodium benzoate was readily soluble in methanol and showed good stability. Hence methanol was selected as solvent.

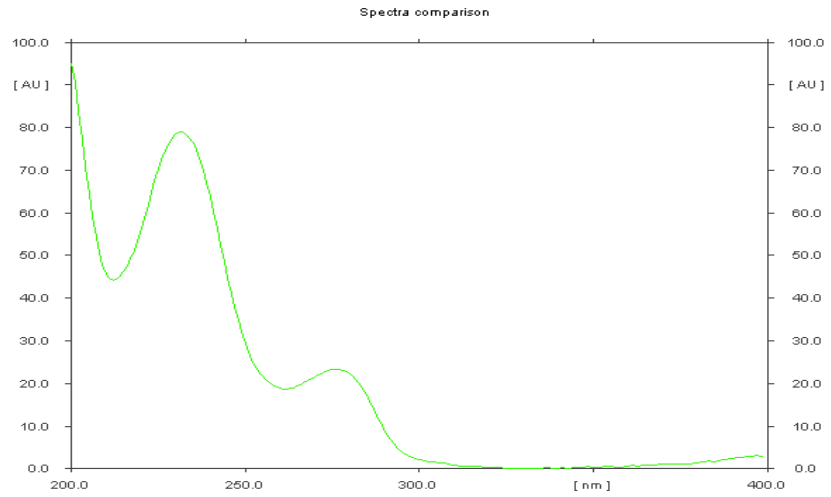
Selection of stationary phase

Based on molecular weight and solubility of sodium benzoate, RP-HPLC method with C_{18} column was employed for the method development.

Selection of wavelength

The wavelength selection was done with the help of UV spectrum of sodium benzoate and it was found that it has maximum absorbance at the wavelength of 232nm. Hence selected for the detection of sodium benzoate and the spectrum is shown in fig 1:

Fig 1: UV spectrum of sodium benzoate



INITIAL CHROMATOGRAPHIC CONDITIONS

Stationary phase : Lichrosphere R 100 RP-18e (5- micron) C18colum

FLOW RATE : 1.0ml/min

Injection volume : 20µl

Operating temperature : Room temperature

Wavelength : 254nm

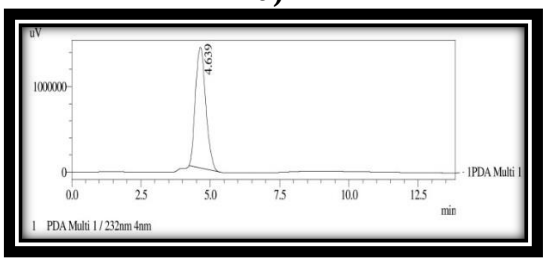
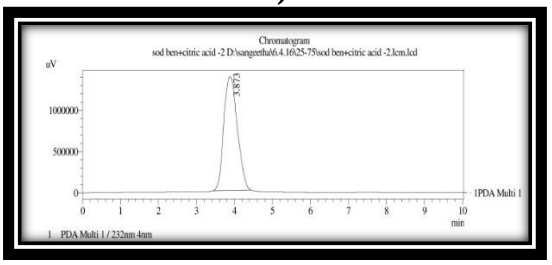
Optimisation of mobile phase

Optimisation of mobile phase was carried out in order to obtain ideal peak of sodium benzoate. The ammonium acetate buffer and methanol resulted in peak with good shape and hence it was selected for further analysis

Effect of ionic strength:

Different strengths of ammonium acetate like 10mM and 20mM were tried. It was found strength of 20mM gave good peak with less tailing. The chromatograms presented in table 1and fig(2)

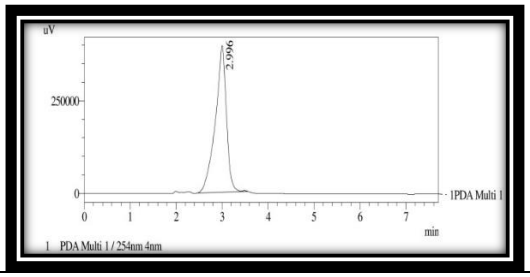
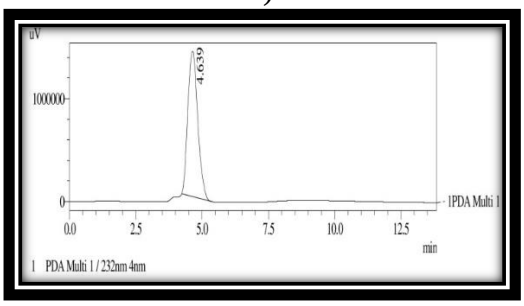
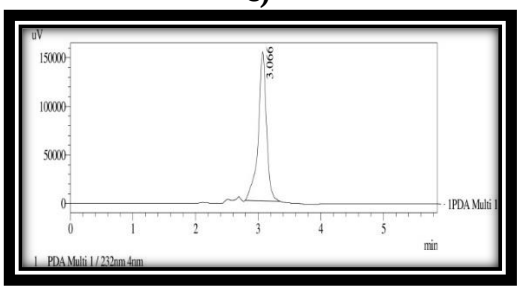
Tab1e 1: Effect of Strength of Ammonium Acetate

Sl.No	Ionic strength	Chromatograms (fig 2)	Observation
1	10mM	**a)** 	Not symmetrical peak
2	20mM	**b)** 	Good peak shape and no tailing

Effect of Ratio

The effect of ratio was studied by varying strength such as 20:80, 35:65 and 25:75(Buffer: methanol %v/v). A ratio of 25:75 %v/v gave good peak characters and hence this ratio was selected for the study. The chromatogram was presented in table 2 and fig (2).

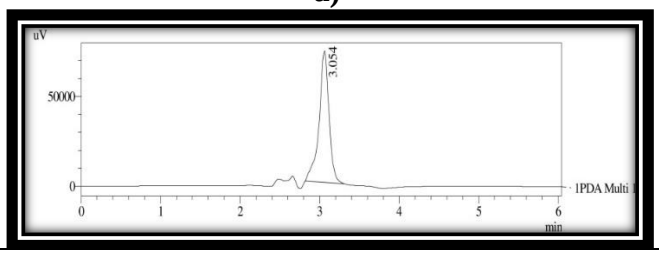
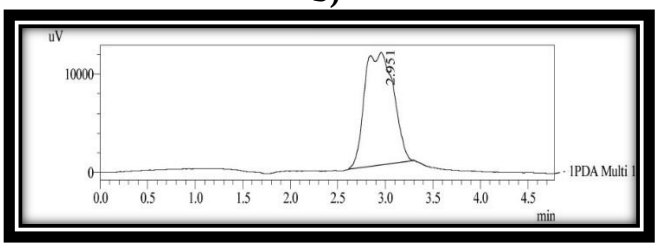
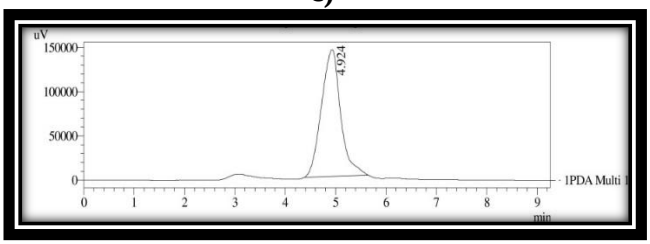
Table 2 : Effect of mobile phase ratio

SI. No	Mobile phase %v/v	Chromatograms (fig 3)	Observation
1	20mM ammonium acetate: methanol (20:80)	a) 	Analyte retention time is less
2	20mM ammonium acetate: methanol (35:65)	b) 	Tailing was occur
3	20mM ammonium acetate: methanol (25:75)	**c)** 	Good peak characteristics.

Effect of pH

Keeping the strength of buffer and ratio constant chromatograms were recorded with different buffer pH like 3.5, 4 and 5. At a pH of 3.5 good symmetrical peaks was obtained and hence it was selected for further studies. The chromatogram is presented in table 3 and fig (4)

Table 3 : Effect of pH

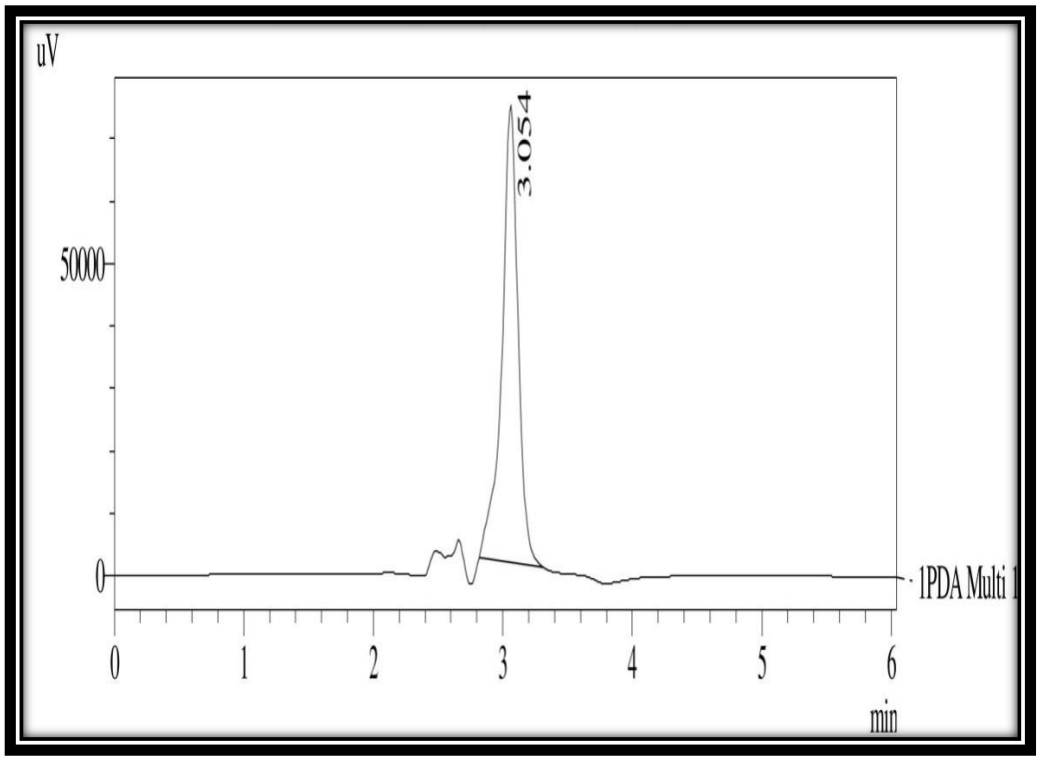
Sl. No	pH	Chromatograms (fig.4)	Observation
1	3.5	**a)** 	Good peak shape and no tailing
2	4	**b)** 	Peak shape is not good
3	5	**c)** 	Tailing was observed

FIXED CHROMATOGRAPHIC CONDITIONS

Stationary phase	:	Lichrosphere R 100 RP-18e (5- micron) C18colum
Mobile phase	:	Ammonium acetate: Methanol (25:75%v/v)
Strength of solvent	:	10 mM
P^H	:	3.50
Detection wavelength	:	232nm
Flow rate	:	1ml/min
Injection volume	:	20µl
Temperature	:	Room temperature
Run time	:	5 min
Retention time	:	3.1 min

The chromatogram of sodium benzoate in fixed chromatographic condition is shown in fig (5)

Fig 5: A chromatogram of sodium benzoate using fixed chromatographic conditions



METHOD VALIDATION

The developed RP-HPLC method was validated according to ICH guidelines in terms of Linearity, accuracy, precision, limit of detection, limit of quantification, robustness and specificity.

LINEARITY AND RANGE

Preparation of standard stock solution

1mg of sodium benzoate was accurately weighed into a 10 ml standard flask and made up to the required with methanol to concentration the 100µg/ml. From 100µg/ml, 5 ml is taken and diluted to 25 ml to get a concentration of 20µg/ml.

Preparation of working standard solution

From 20µg/ml, 1-5ml was taken and diluted to 10 ml to get a series of concentration of 2-10µg/ml.

Recording of a standard chromatogram

In the Schimadzu HPLC System, the chromatographic conditions were maintained. After a base line was obtained, 20µl of the prepared working standard solution was injected and the chromatograms were recorded. The peak areas of these solutions were noted at 232nm. A calibration graph was plotted with measured concentration against peak area.

Precision

It was evaluated by repeatability, intra-day precision and inter- day precision. Intra-day precision was determined by injecting standard solution in between linearity range three times on the same day. Inter- day precision was found by repeating analysis on three consecutive days. Repeatability of injection was determined by injecting standard solutions for six times. The peak areas were noted and %RSD was calculated.

LOD and LOQ

LOD and LOQ were calculated in terms of signal to noise ratio. LOD is the lowest concentration of the analyte that can produce a response detectable above the noise level of the system, typically three times the noise level.

S/N=2/1 (or) 3/1

LOQ is the lowest level of the analyte that can accurately and precisely measured.

Stability

Sample solution Sample solution of sodium benzoate was subjected to stability studies under room temperature. Stabilities were studied by looking for any change in retention time, and peak shape when compared to chromatogram of freshly prepared solution.

Robustness

In order to demonstrate the robustness of the method, the following optimised conditions were slightly varied and the effect on peak areas was noted.

- ± 2% in ratios of methanol in mobile phase.
- ± 0.1 ml flow rate

Specificity

The solvent and mobile phase were injected into fixed chromatographic system and observed for any additional peaks to assess specification of the method.

System suitability parameters:

The system suitability parameters like tailing factor, theoretical plate and As-symmetric factor were calculated from the standard chromatograms.

EXTRACTION OF SODIUM BENZOATE FROM SOFT DRINKS AND FRUIT JUICES

EXTRACTION PROCEDURE

In order to extract sodium benzoate from selected food materials the following were carried out.

MIRINDA:

To extract sodium benzoate from mirinda, 10ml of mirinda was taken in a 25 ml standard flask and it was shaken to degas from this 1 ml was taken and added with 1ml of methanol and injected.

7UP

To extract sodium benzoate from 7up, 10ml of 7up was taken in a 25 ml standard flask and it was shaken to degas and from that 1 ml was taken and it was added with 1ml of methanol and injected.

REVIVE HYDROTONIC DRINK

To extract sodium benzoate from revive,10ml revive of was taken in a 25ml standard flask and it was shaken to degas and from that 1 ml was taken and it was added with 1ml of methanol and injected.

EXTRACTION OF SODIUM BENZOATE FROM FRUIT JUICES

EXTRACTION PROCEDURE

FROOTI

To extract sodium benzoate from frooti, 4ml of frooti and 8ml of ether was taken in a separating funnel and it was shaken thoroughly. The organic layer was separated and evaporated. After cooling 1 ml methanol was added to reconstitute and injected.

MAA

To extract sodium benzoate from maa, 4ml of maa and 8ml of ether was taken in a separating funnel and it was shaken well. The organic layer was separated and evaporated . After cooling 1 ml methanol was added to reconstitute and injected.

Analysis of extracted sodium benzoate by HPLC

All the extracted samples from mirinda, 7UP, revive, frooti and maa were injected into the HPLC system. The peak areas were noted and the amount of sodium benzoate present in each samples was calculated using calibration graph.

RECOVERY STUDIES

Recovery studies of soft drinks and fruit juices were carried out to prove the accuracy of the method.

PROCEDURE

Soft Drinks

To soft drinks, standard sodium benzoate was added to sample and shaken well. From this 1ml was taken and diluted with 1ml of methanol and injected.

Fruit Juices

To fruit juices, standard sodium benzoate was added to sample and shaken well. It was extracted with ether, after separating organic layer, evaporation was done. After an appropriate reconstitution it was analysed. Amount of sodium benzoate extracted before and after addition of standard was calculated to prove the accuracy of the method.

RESULTS AND DISCUSSION

Method Development

A RP-HPLC method was developed where optimisation of mobile phase conditions were carried out to achieve ideal peak for sodium benzoate. Among Ammonium acetate buffer was chosen along with methanol organic phase. Among different ionic strength tried 20mM chosen as others resulted in broad peaks. In the ratio of 25:75 (A: B, buffer: Methanol) peak was good. pH of buffer above and below 3.5 resulted in peak broadening and hence 3.5 was fixed. Flow rate of 1ml/min was found ideal.

Linearity:

From the linearity graph it was found that sodium benzoate showed good linearity in the concentration range of 2-10 mcg/ml. The standard chromatograms are shown in fig 6 to 10. The calibration data of sodium benzoate graph is represented in fig 11 and table-4.

STANDARD CHROMATOGRAMS

Fig 6 : standard chromatogram of sodium benzoate 2µg/ml

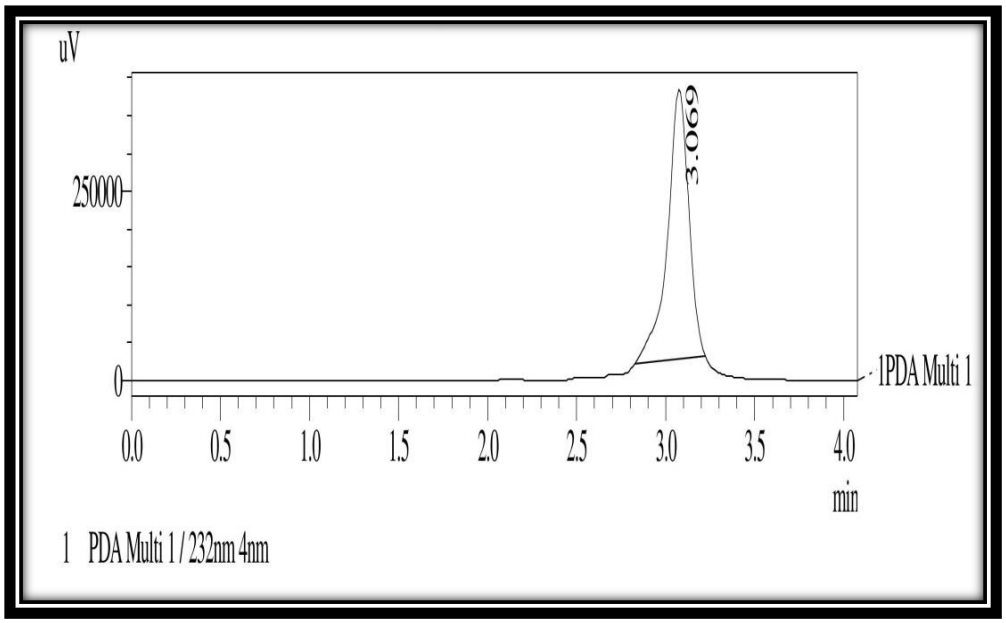


Fig 7: standard chromatogram of sodium benzoate 4µg/ml

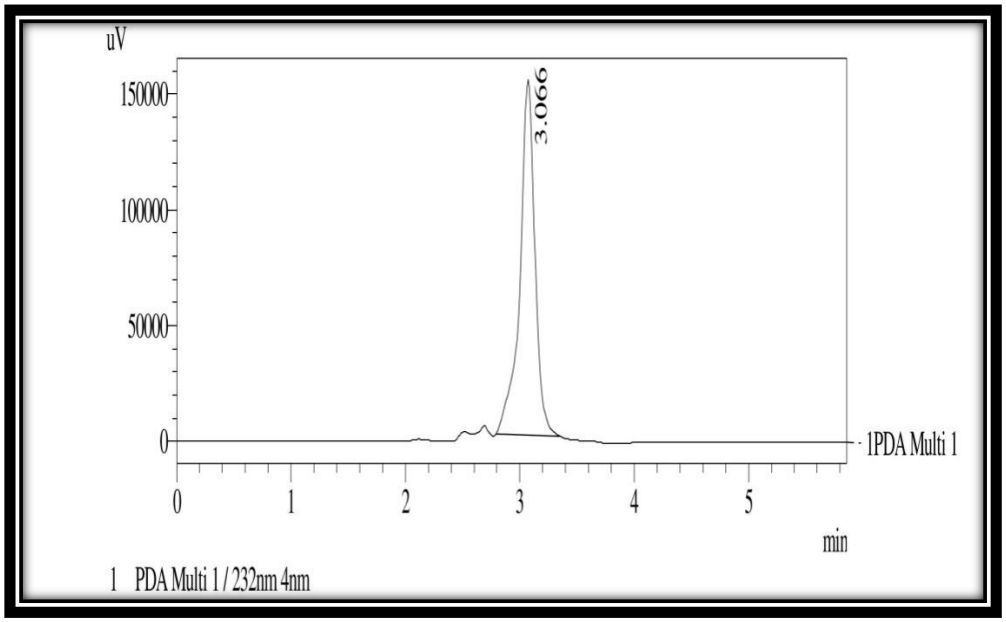


Fig 8 : standard chromatogram of sodium benzoate 6µg/ml

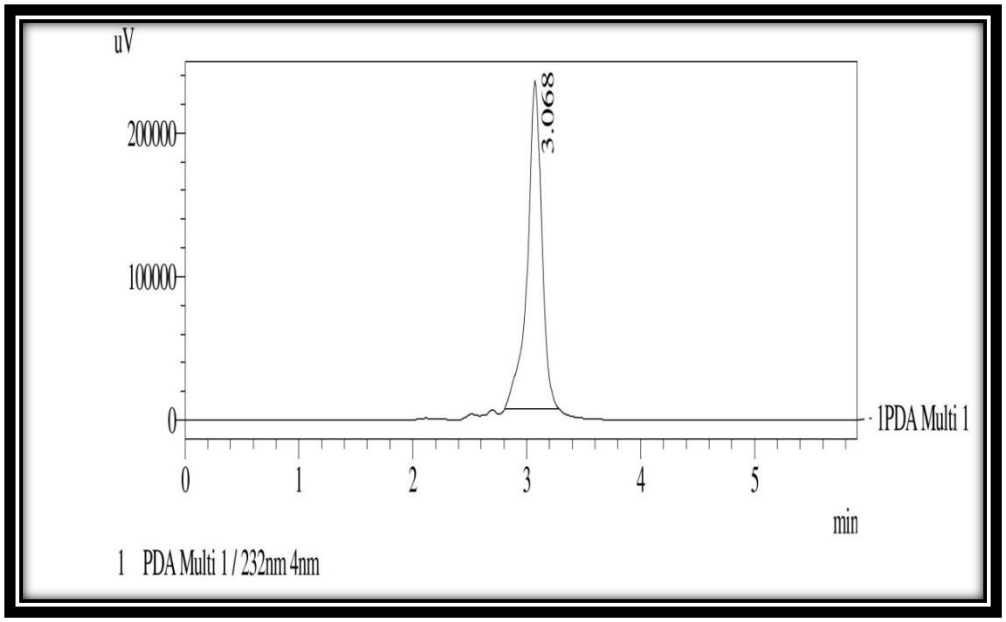


Fig 9 : standard chromatogram of sodium benzoate 8µg/ml

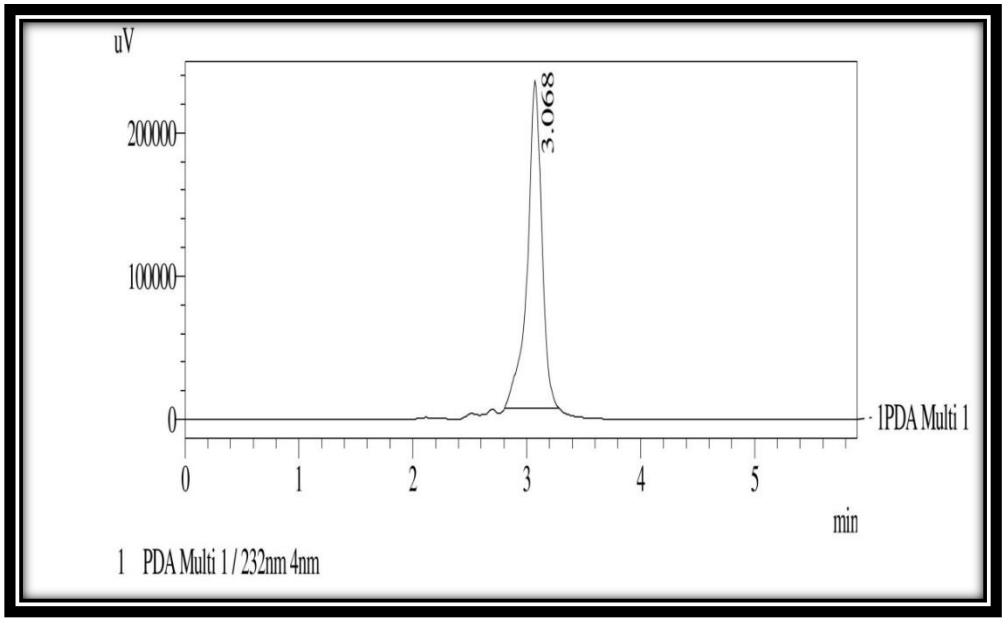


Fig10 : standard chromatogram of sodium benzoate 10µg/ml

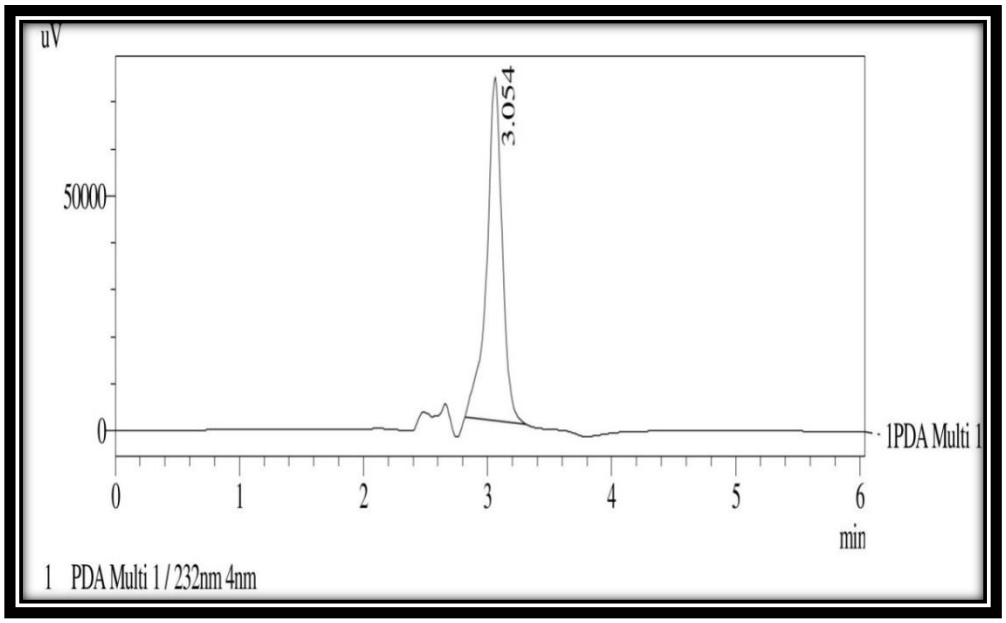


Fig 11: Calibration graph

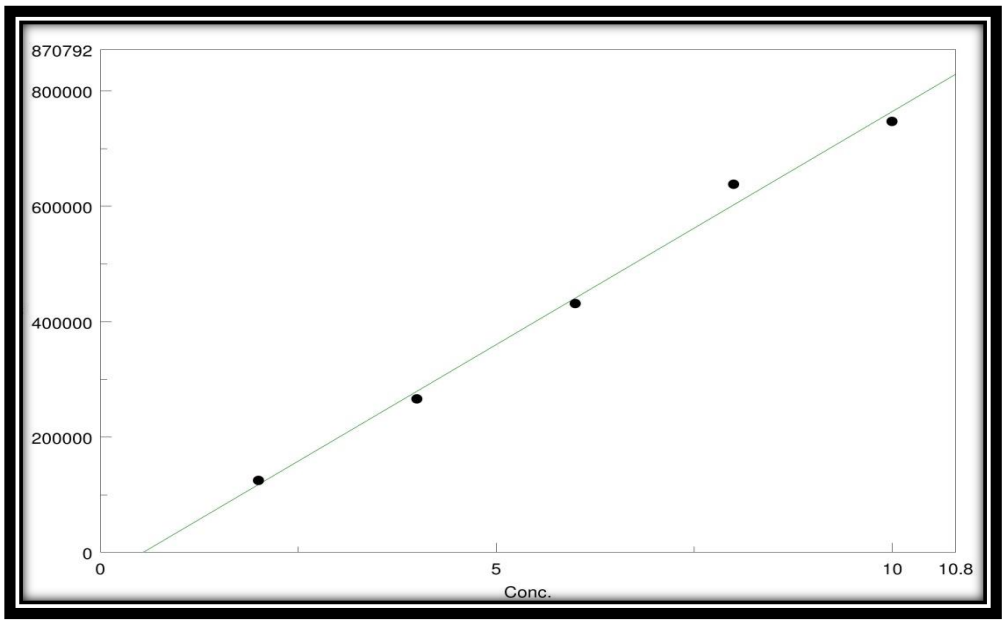


Table 4: Calibration data

Concentration(mcg/ml)	Peak area
2	124996
4	265918
6	431053
8	637769
10	746493

Precision

Precision of the method was determined by performing repeatability, intra day and inter-day precision studies.

1. Repeatability

Repeatability of injection was determined by injecting standard solutions(6mcg/ml) six times and % RSD was calculated table-5

Table 5 : Repeatability studies

Concentration(mcg/ml)	Peak area	%RSD
6	431053	0.134
	431156	
	432015	
	432256	
	432265	
	432300	

2. Intra-day precision

Intra-day precision was determined by injecting standard solutions of (4 and 6mcg/ml) for three times on the same day and %RSD was calculated, table.6.

Table 6 : Intra-day precision studies

Concentration(mcg/ml)	Peak area	%RSD
4	264896	0.025
	264893	
	264906	
6	431962	0.018
	432013	
	432116	

3. Inter-day precision

Inter-day precision was determined by injecting standard solutions (4 and 6mcg/ml) for three days and % RSD was calculated, table.7.

Table 7: Inter-day precision studies

Concentration(mcg/ml)	Peak area	%RSD
4	265156	0.009
	265185	
	265204	
6	431146	0.16
	432145	
	432546	

4. LOD and LOQ:

LOD and LOQ values were found to be 0.0001, 0.0003 mcg/ml and mcg/ml, respectively.

Table 8.System suitability parameters

Tailing factor	Theoretical plate	Asymmetric factor
0.9	2068.6	1.0

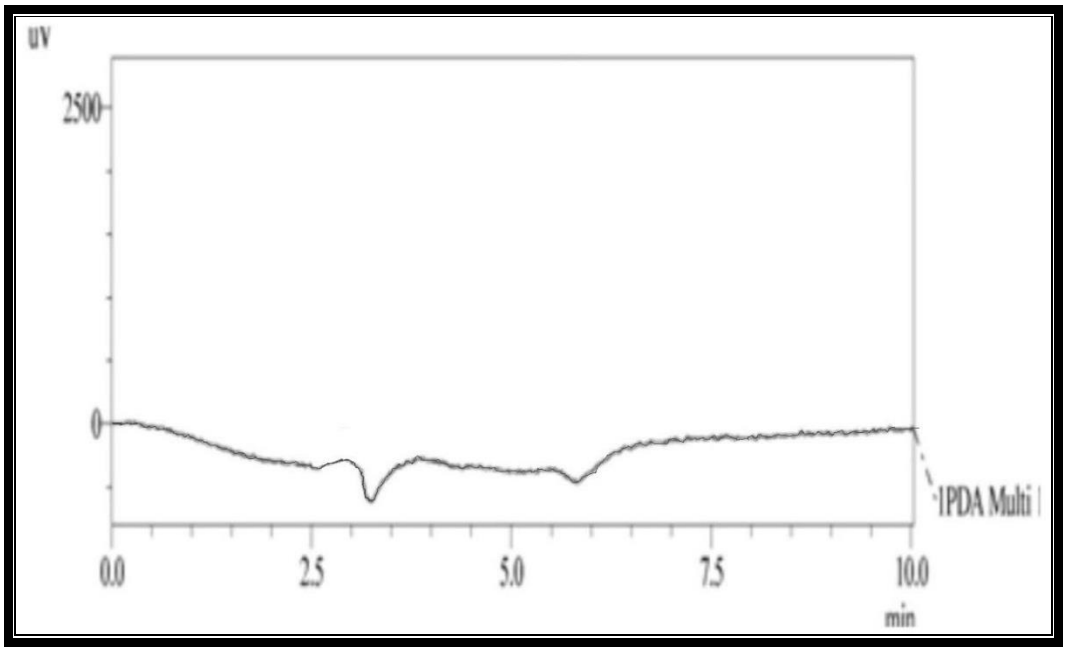
5. Robustness

The response factor peak areas for the changed chromatographic parameters (%B and flow rate) were almost same as that of the fixed chromatographic parameters and hence the developed method is said to be robust.

6. Specificity

There were no additional peaks observed while injecting solvents or mobile phase alone. This proves specificity of the method. The chromatogram is shown in figure 12.

Fig 12. Blank Chromatogram



7. Stability of solution:

The solution under room temperature was stable for 36 hours and up to 48 hours stable under refrigeration.

QUANTIFICATION OF SODIUM BENZOATE FROM SOFT DRINKS AND FRUIT JUICES BY DEVELOPED HPLC METHOD

The Quantification of sodium benzoate from soft drinks and fruit juices was carried out after proper dilution / extraction. Soft drinks were first degassed and diluted with methanol before analysis. The fruit juices were extracted with ether, after removal of ether reconstituted with methanol before analysis.

Quantification of sodium benzoate from mirinda

Three samples of mirinda was analysed and the chromatograms are shown in fig below (13,15,17). The peak purity index value is close 1 shows that sodium benzoate is extracted matching with that of standard and it is specific. From the peak areas noted the amount present in samples were calculated and shown in table 9 and spectra shown in fig 14,16,18.

Fig 13. Chromatogram of sodium benzoate extracted from Mirinda1:

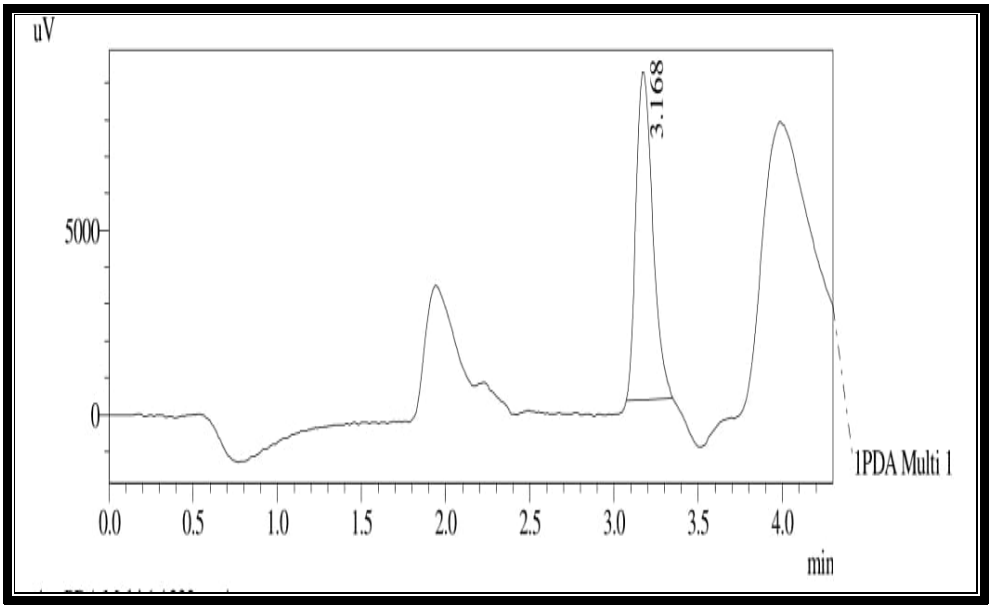


Fig 14. Spectra of sodium benzoate extracted from Mirinda1

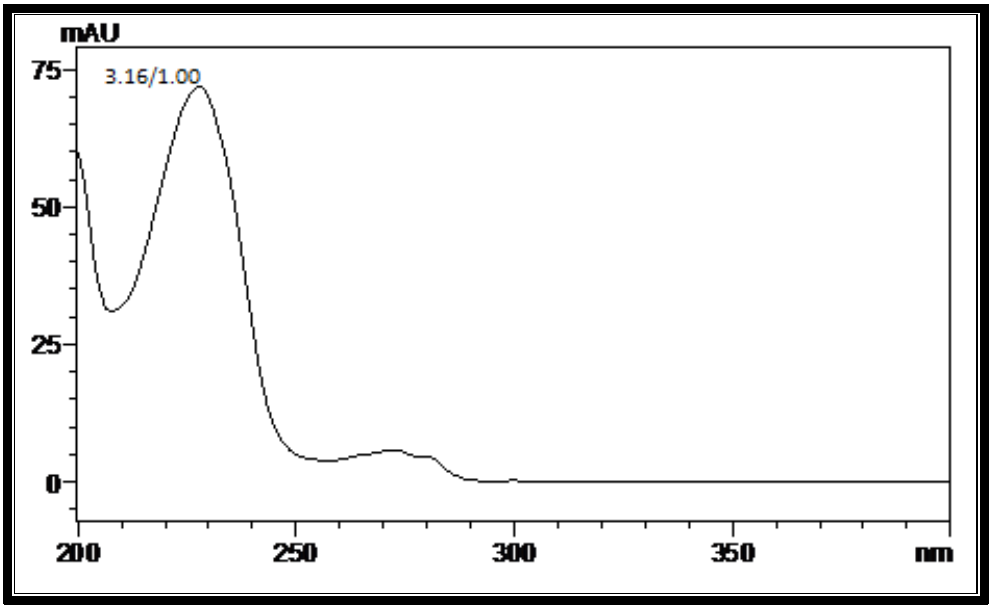


Fig 15. Chromatogram of sodium benzoate extracted from Mirinda 2

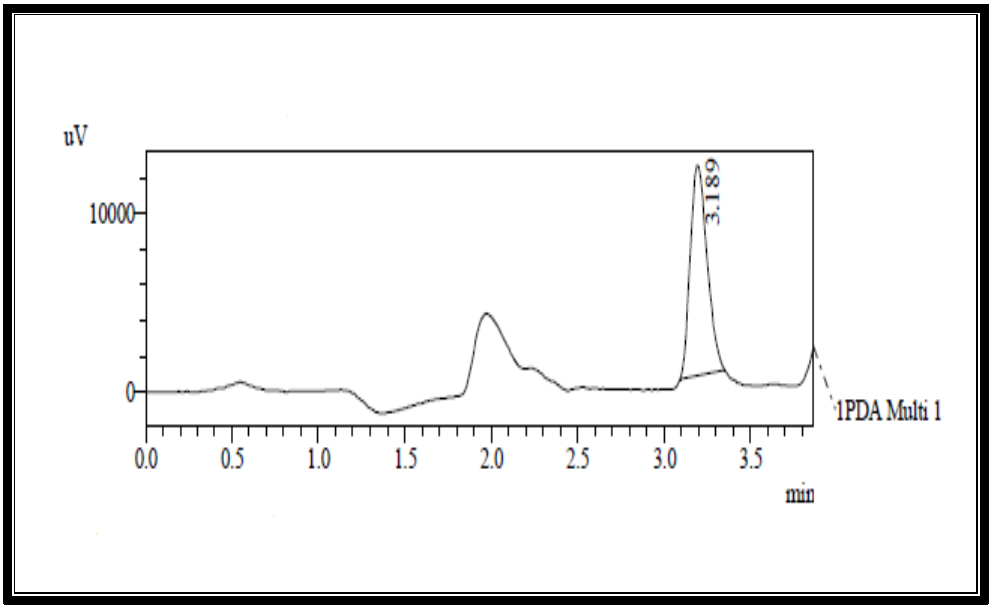


Fig 16. Spectra of sodium benzoate extracted from Mirinda 2

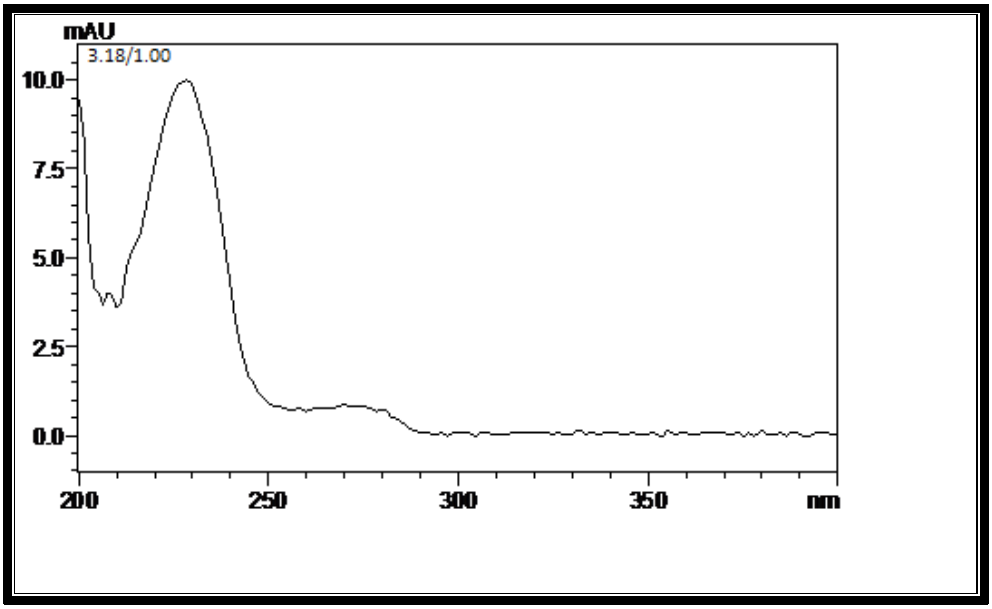


Fig 17. Chromatogram of sodium benzoate extracted from Mirinda 3

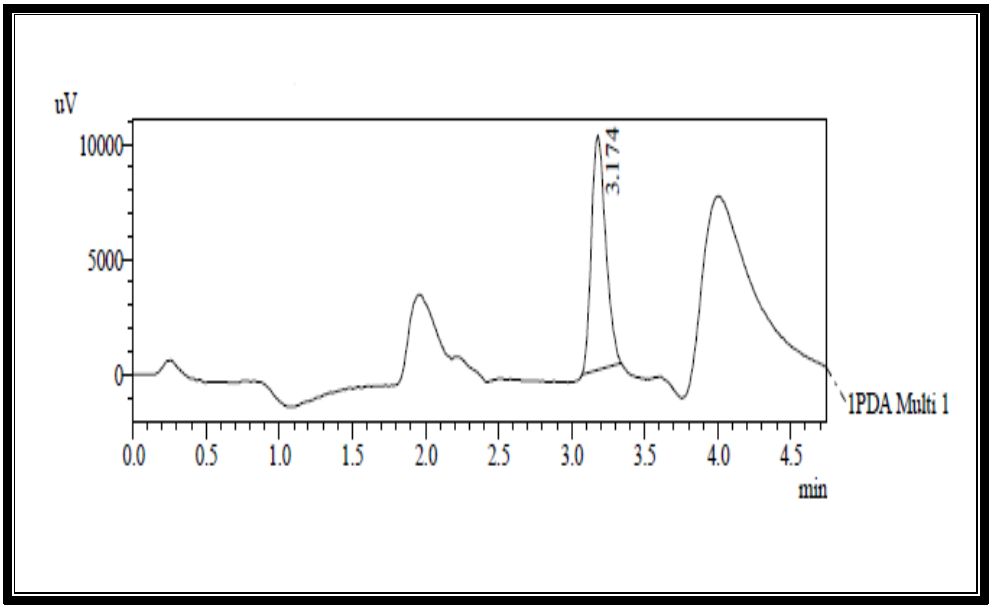


Fig 18. Spectra of sodium benzoate extracted from Mirinda 3

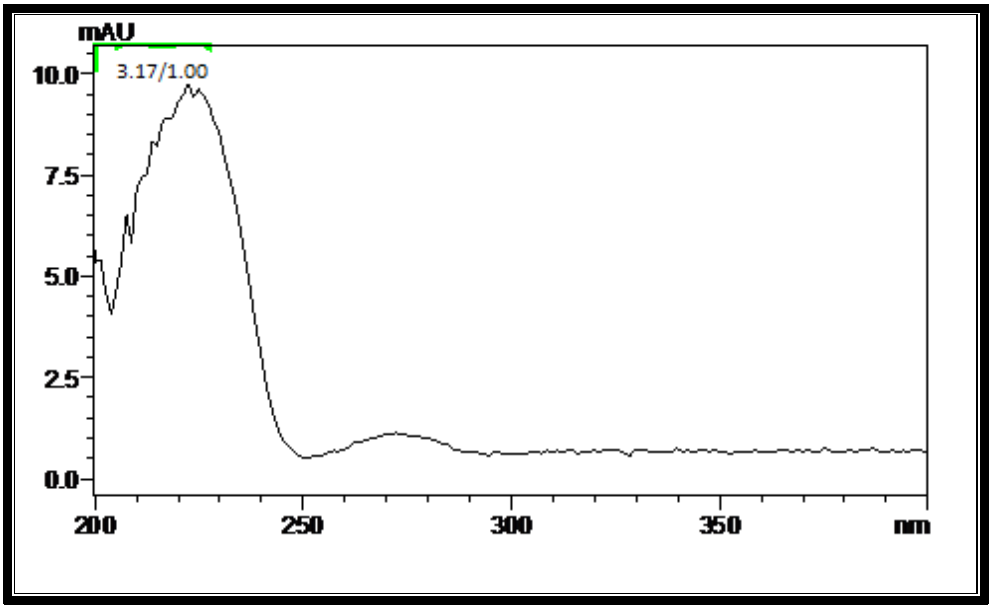


TABLE 9. Results of analysis of Mirinda for sodium benzoate

S.no	Product	Peak area	Concentration (μg/10ml)	Present in 600 ml(μg)
1.	MIRINDA-1	61847	1.30	78.0
		69817	1.40	84.0
		64987	1.34	80.4
2.	MIRINDA-2	80266	1.53	91.8
		82316	1.55	93.0
		77210	1.49	89.4
3.	MIRINDA-3	99697	1.77	106.2
		98429	1.75	105.0
		96521	1.73	103.8
			Average	92.4 μg

QUANTIFICATION OF SODIUM BENZOATE FROM 7UP BY DEVELOPED METHOD

The 7up samples (3 batches) were degassed, diluted and injected to record chromatograms. They are shown in fig 19,21,23. The spectra of sodium benzoate extracted was compared with standard and spectra and found to be matching. The peak purity index value is of 0.9999 shows the specificity of peak for sodium benzoate.

The peak areas noted and amount of sodium benzoate calculated are presented in table 10 and respective spectrum shown in fig 20,22,24.

Fig.19 chromatogram of sodium benzoate extracted from 7up(1)

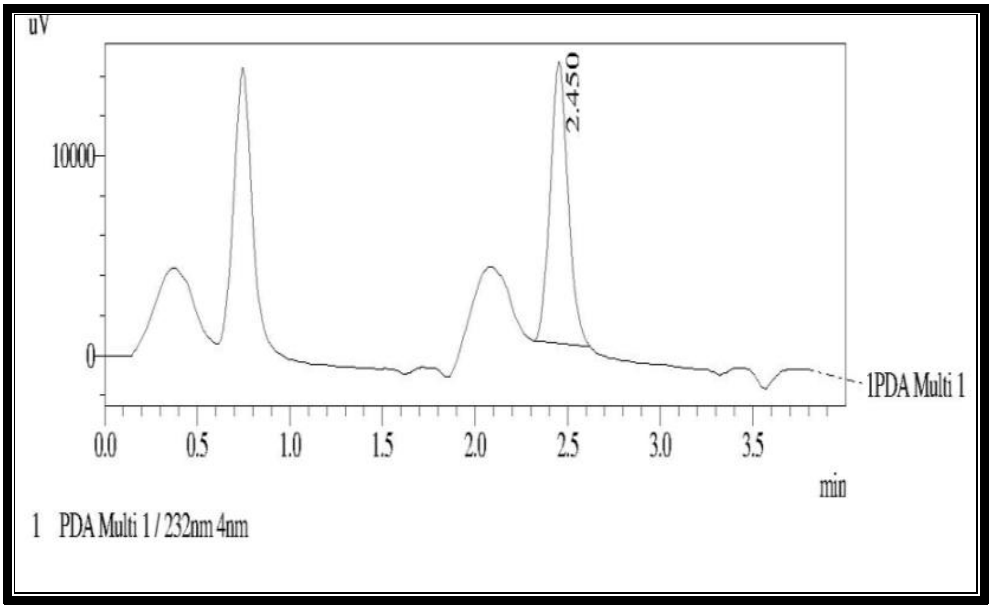


Fig 20. Spectrum of sodium benzoate extracted from 7up(1)

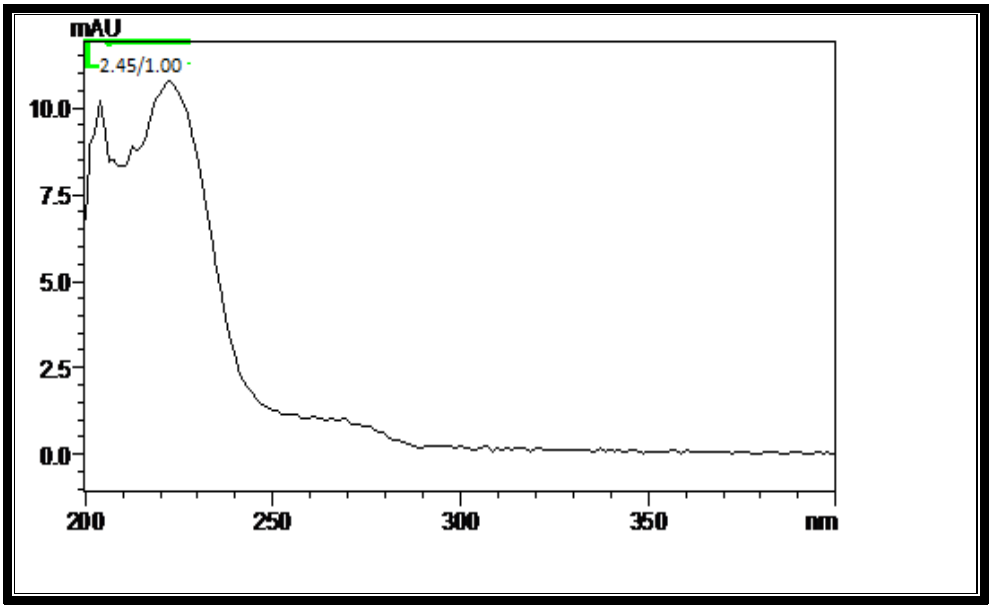


Fig.21 chromatogram of sodium benzoate extracted from 7up(2)

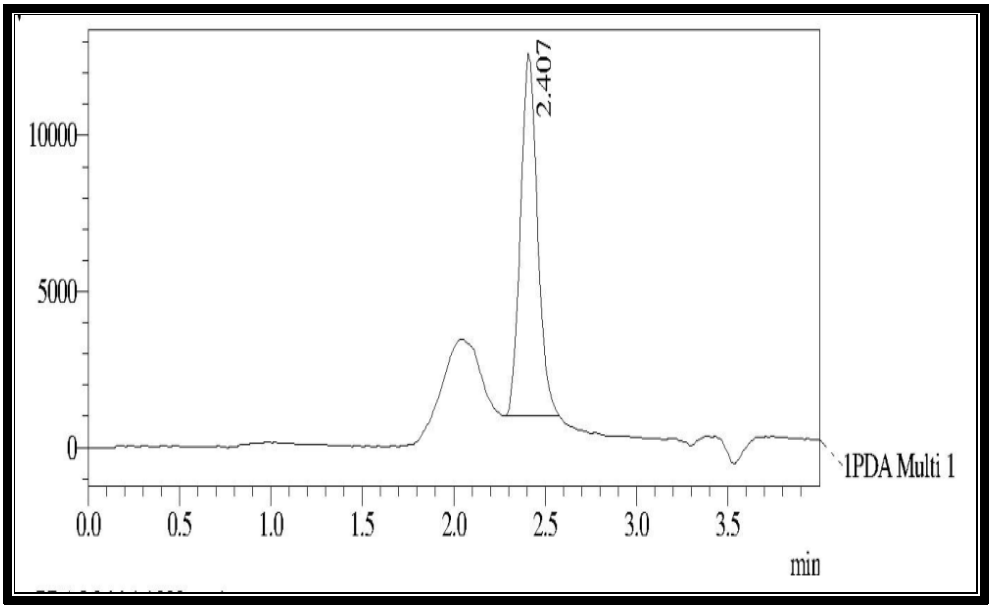


Fig. 22. Spectrum of sodium benzoate extracted from 7up(2)

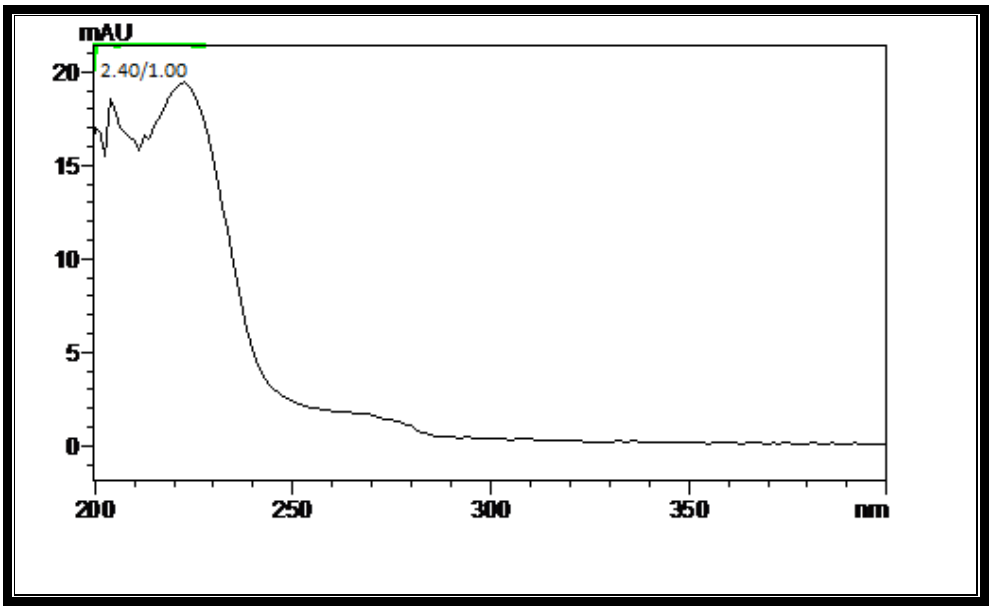


Fig. 23 chromatogram of sodium benzoate extracted from 7up(3)

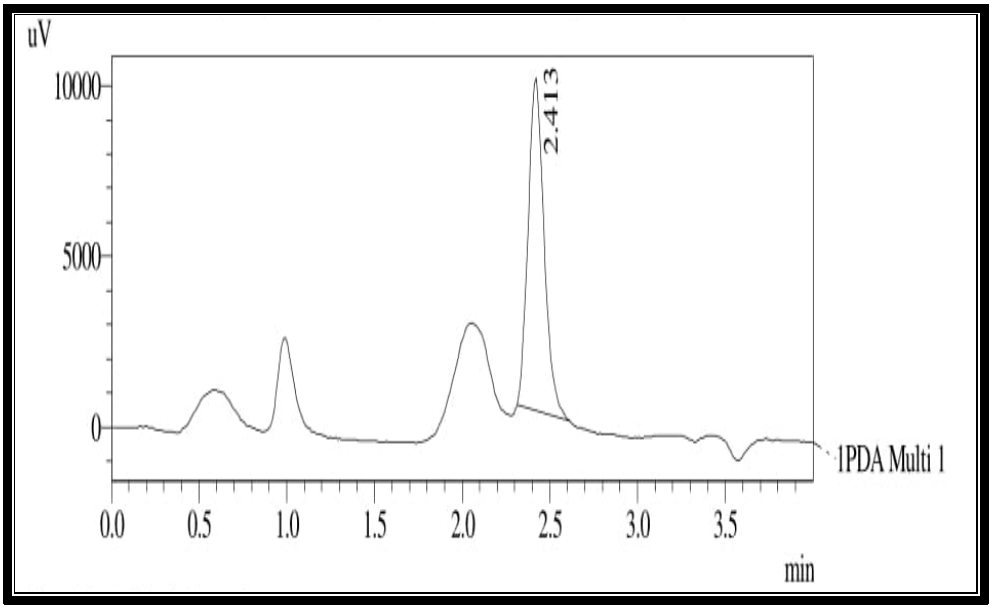


Fig 24. Spectrum of sodium benzoate extracted from 7up(3)

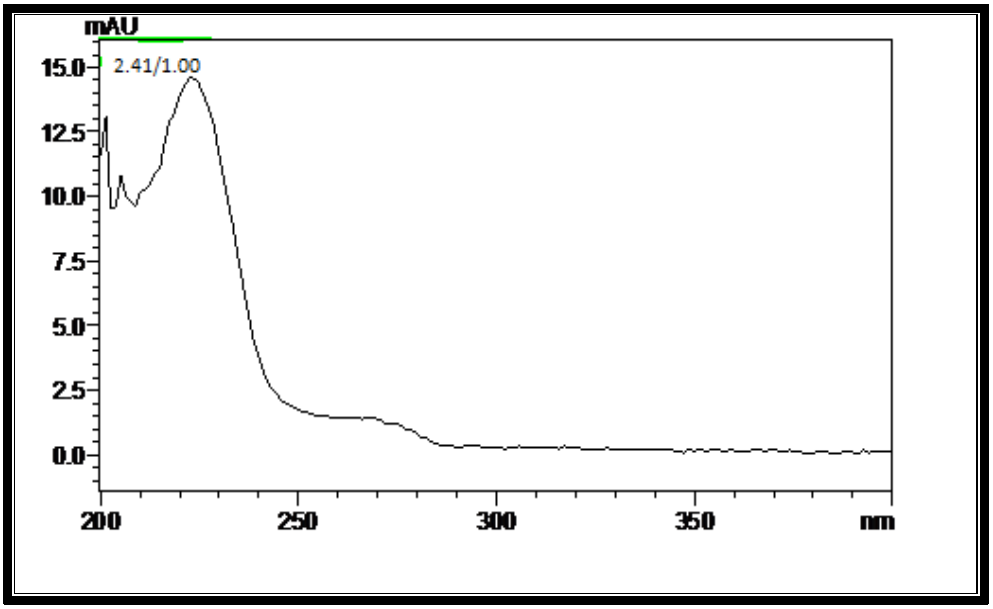


Table 10 : Results of analysis of 7up for sodium benzoate

S.no	Product	Peak area	Concentration (μg/10ml)	Present in 600 ml(μg)
1	**7UP-1**	74830	1.46	87.6
		73299	1.44	86.4
		63430	1.32	79.2
2.	**7UP-2**	94200	1.70	102.0
		82179	1.55	93.0
		83694	1.57	94.2
3.	**7UP-3**	88385	1.63	97.8
		87275	1.62	97.2
		85121	1.59	95.4
			Average	92.5 μg

QUANTIFICATION OF SODIUM BENZOATE IN REVIVE HYDROTONIC DRINK BY DEVELOPED METHOD

Sodium benzoate was extracted from revive hydrotonic drink by simple dilution method. The chromatograms are presented in fig 25,27,29 and spectra shown in fig. 26, 28, 30.The spectra was matching with standard sodium benzoate and the peak purity was close to 1.The result of analysis of samples represented in table 11.

Fig. 25. Chromatogram of Sodium Benzoate extracted from Revive Hydrotonic Drink(1)

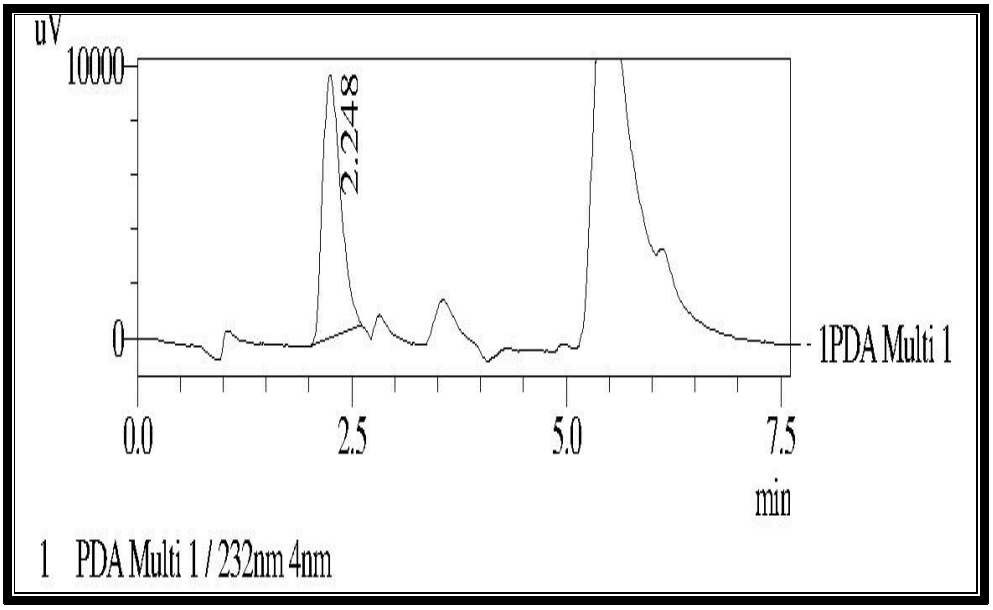


Fig.26. Spectrum of Sodium Benzoate Extracted from Revive Hydrotonic Drink(1)

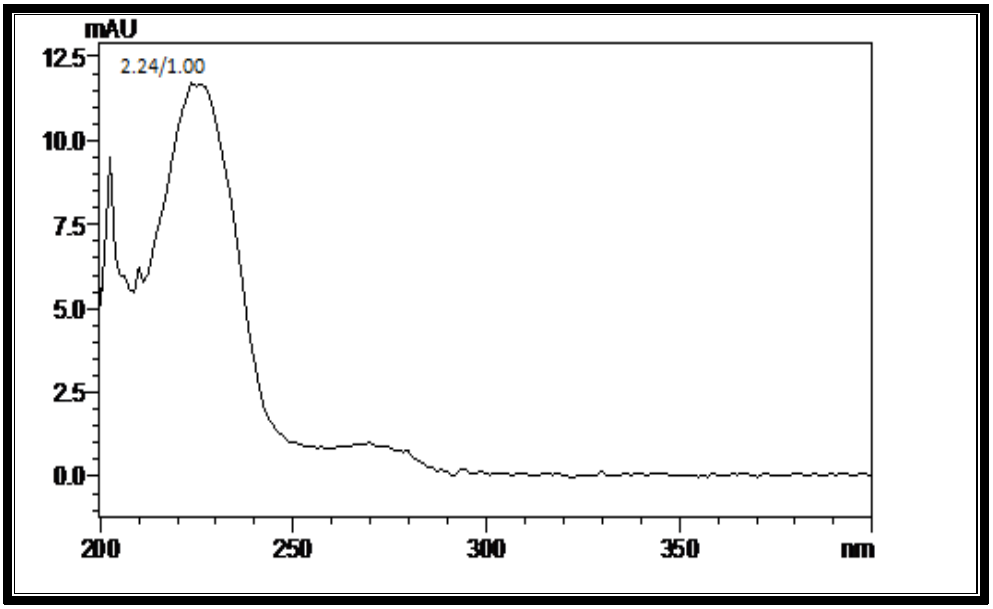


Fig 27. Chromatogram of Sodium Benzoate extracted from Revive Hydrotonic Drink(2)

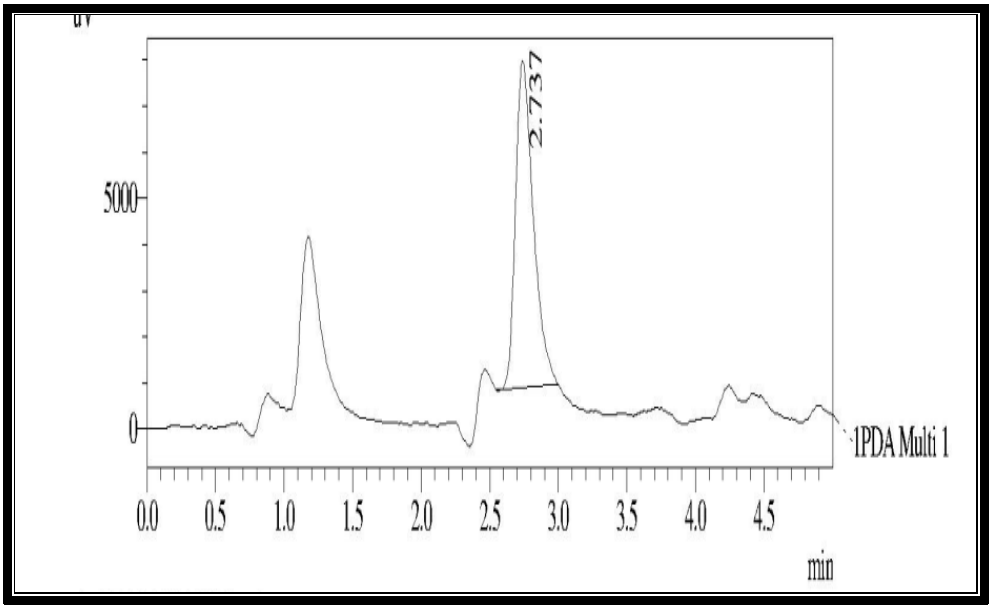


Fig 28.Spectrum of Sodium Benzoate Extracted from Revive Hydrotonic Drink(2)

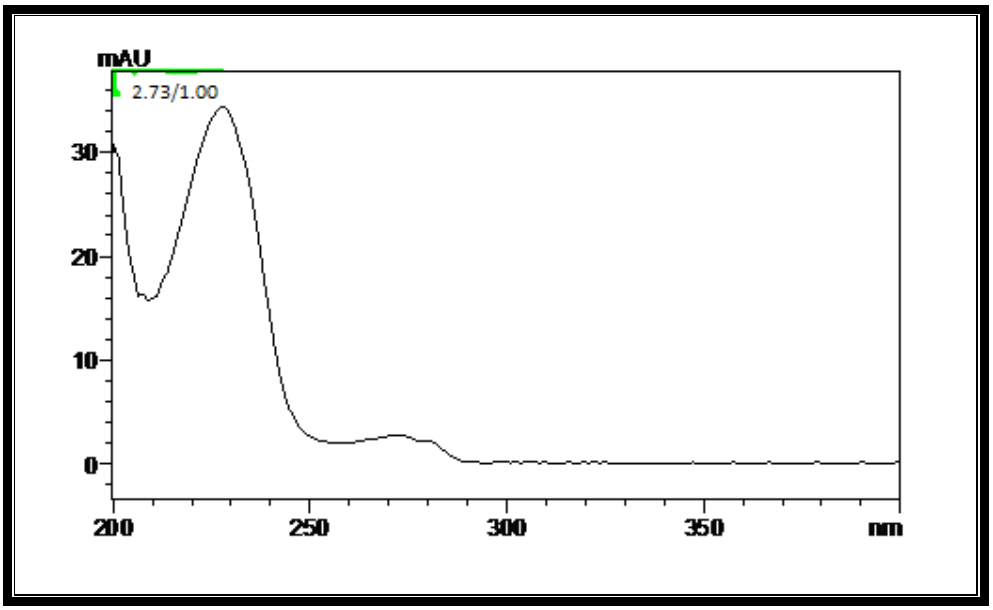


Fig 29.Chromatogram of Sodium Benzoate Extracted From Revive Hydrotonic Drink(3)

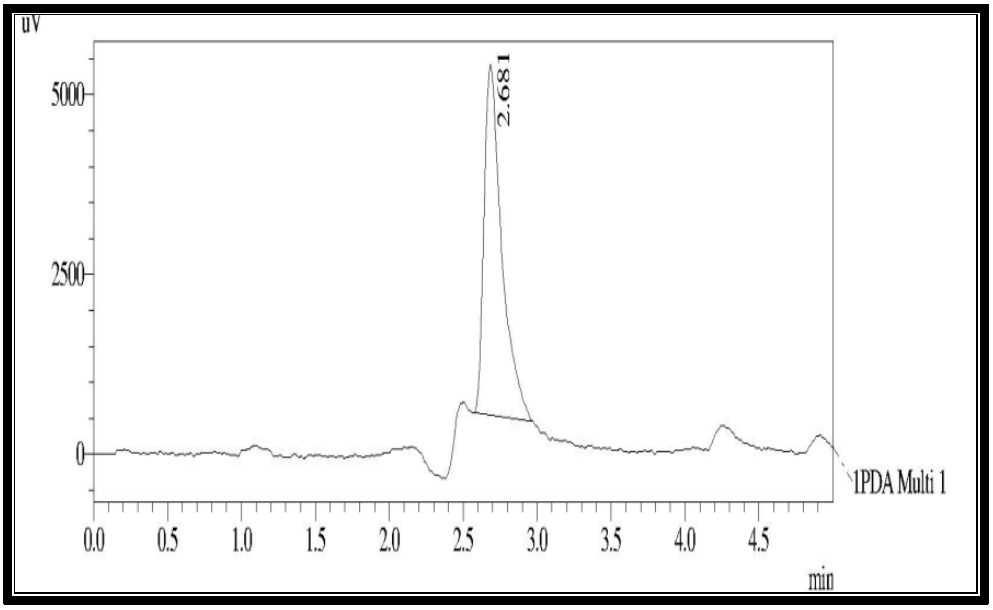


Fig. 30. Spectrum of Sodium Benzoate Extracted from Revive Hydrotonic Drink(3)

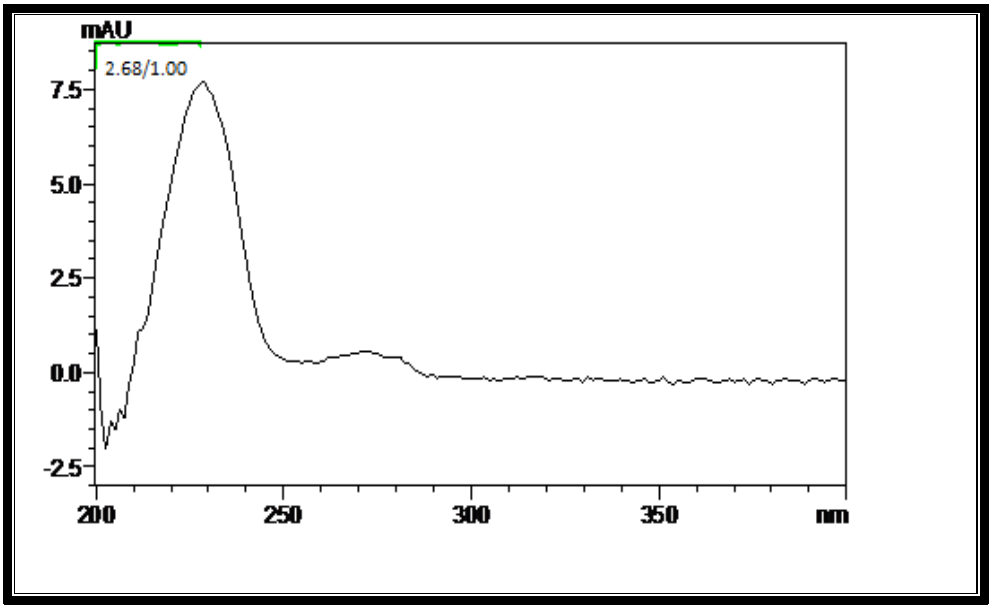


Table 11. Results and analysis of revive hydrotonic drink for sodium benzoate

S.No	Product	Peak area	Concentration (μg/10ml)	Present in 330 ml(μg)
1	HYDROTONIC REVIVE DRINK -1	40670	1.04	34.32
		42593	1.06	34.98
		46394	1.11	36.63
2	HYDROTONIC REVIVE DRINK -2	63864	1.33	43.89
		59642	1.27	41.91
		59624	1.27	41.91
3	HYDROTONIC REVIVE DRINK -3	77576	1.50	49.5
		74124	1.45	47.85
		78569	1.51	49.83
			Average	42.31 μg

QUANTIFICATION OF SODIUM BENZOATE IN FROOTI BY DEVELOPED HPLC METHOD

The chromatograms of sodium benzoate extracted from frooti shown in fig. 31,33,35 and spectra shown in fig. 32,34,36.The results of analysis of frooti samples for sodium benzoate shown in table 12.

Fig 31.Chromatogram of sodium benzoate extracted from Frooti (1):

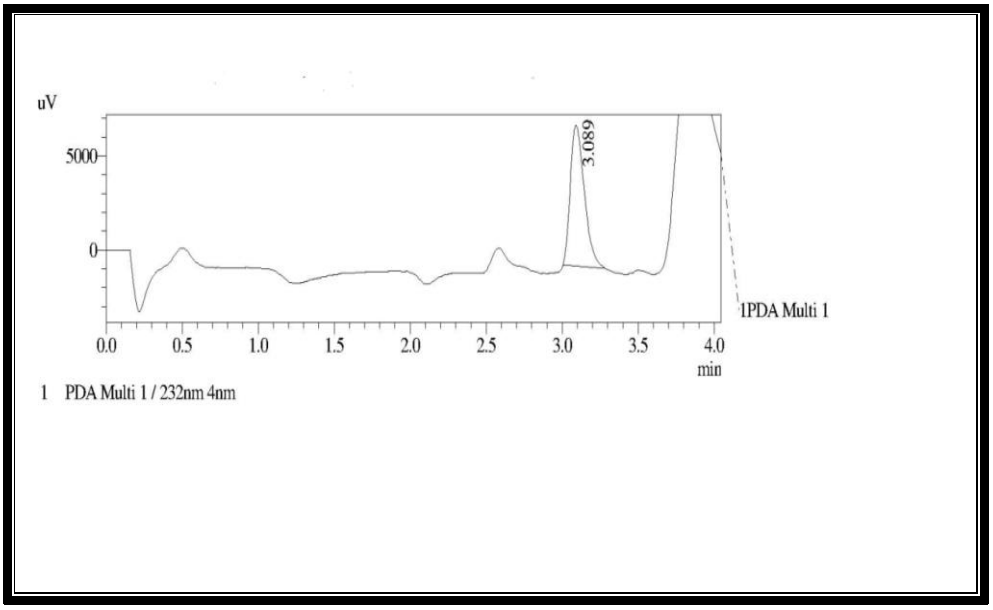


Fig 32. Spectrum of sodium benzoate extracted from Frooti (1)

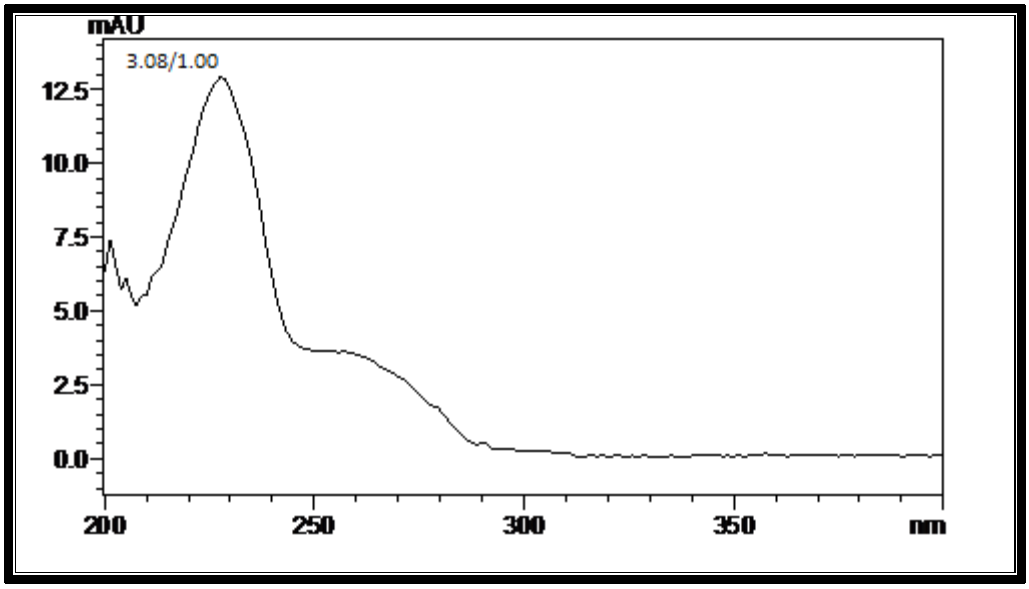


Fig 33.Chromatogram of sodium benzoate extracted from Frooti (2)

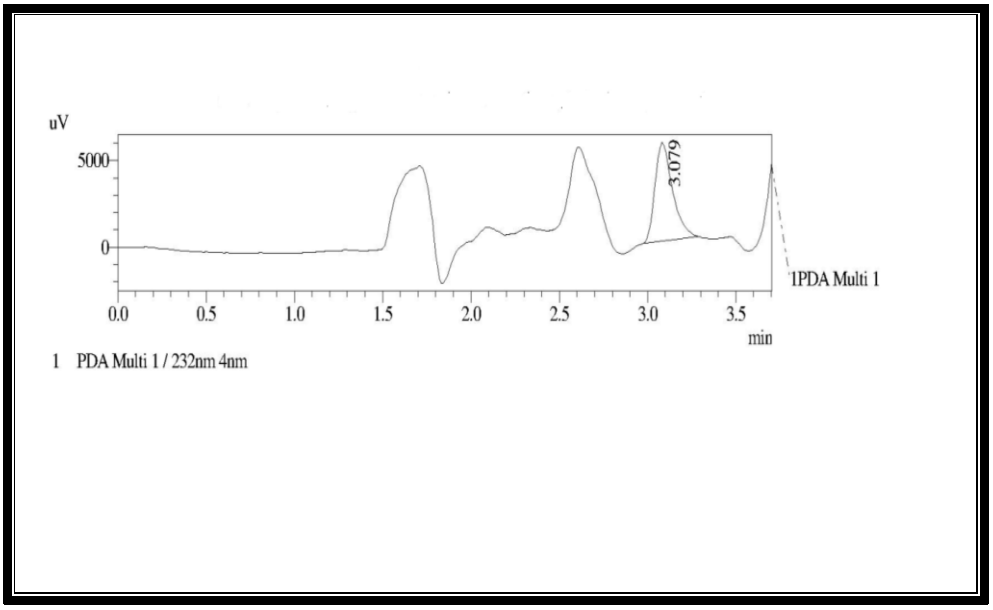


Fig 34. Spectrum of sodium benzoate extracted from Frooti (2)

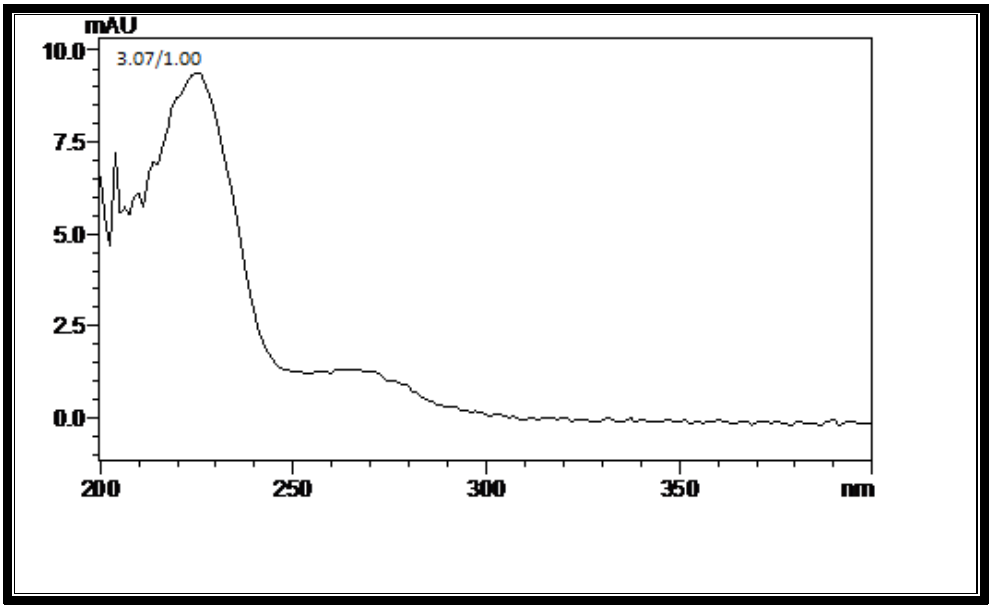


Fig 35.Chromatogram of sodium benzoate extracted from Frooti (3)

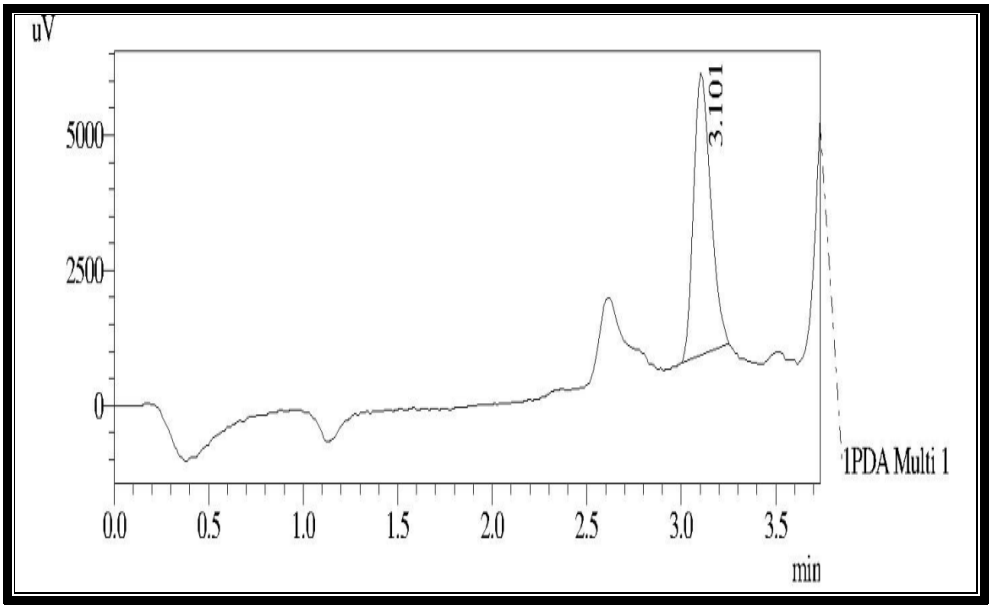


Fig 36. Spectrum of sodium benzoate extracted from frooti (3)

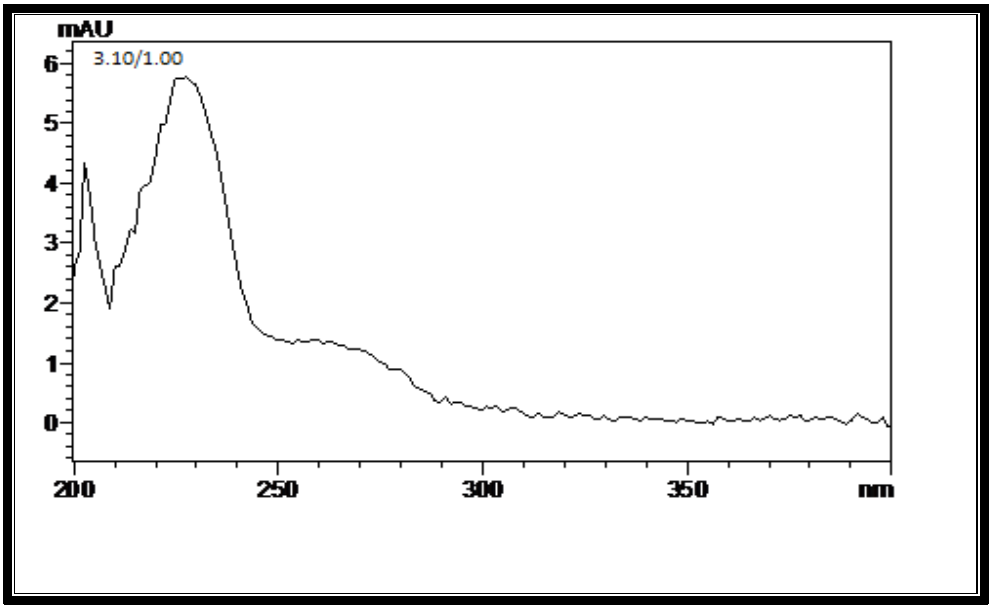


Table 12. Results and analysis of frooti for sodium benzoate

S.No	Product	Peak area	Concentration (μg/4ml)	Present in 600ml(μg)
1	FROOTI-1	27576	0.88	132.0
		22584	0.81	121.5
		32568	0.94	141.0
2	FROOTI-2	33104	0.95	142.5
		45891	1.10	165.0
		47548	1.12	168.0
3	FROOTI-3	41667	1.05	157.5
		47548	1.12	168.0
		58336	1.26	189.0
			Average	153.8 μg

QUANTIFICATION OF SODIUM BENZOATE IN MAA BY DEVELOPED HPLC METHOD

The different batch Maa samples were analysed for sodium benzoate after extract ion with ether. The chromatograms are shown in fig. 37,39,41 and spectra shown in fig 38,40,42. The amount of sodium benzoate calculated from peak areas is shown in table 13.

The peak purity index value and spectra confirm the presence of sodium benzoate in maa samples.

Fig. 37. Chromatogram of sodium benzoate extracted from MAA(1):

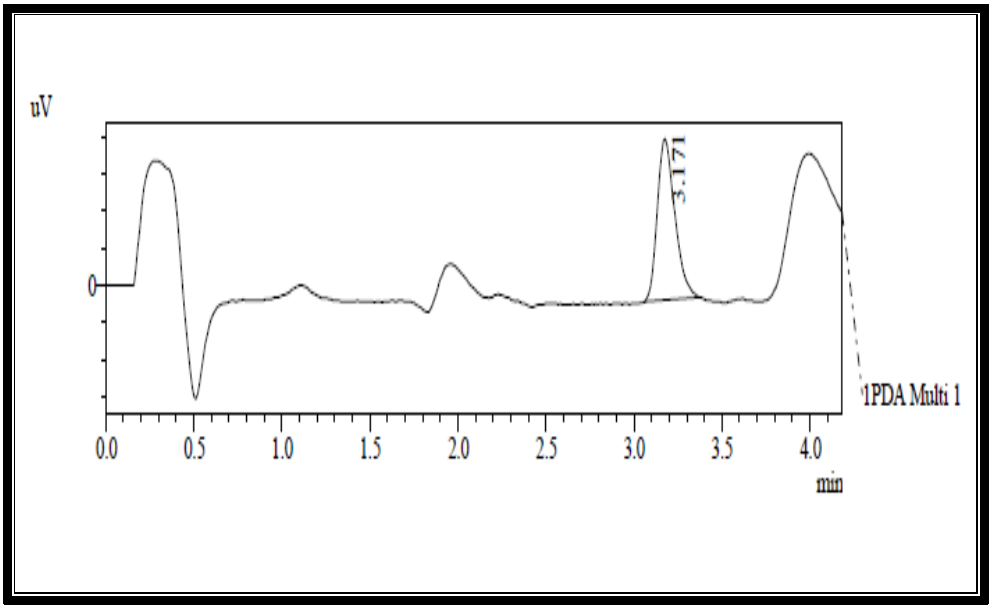


Fig. 38. Spectrum of sodium benzoate extracted from Maa (1)

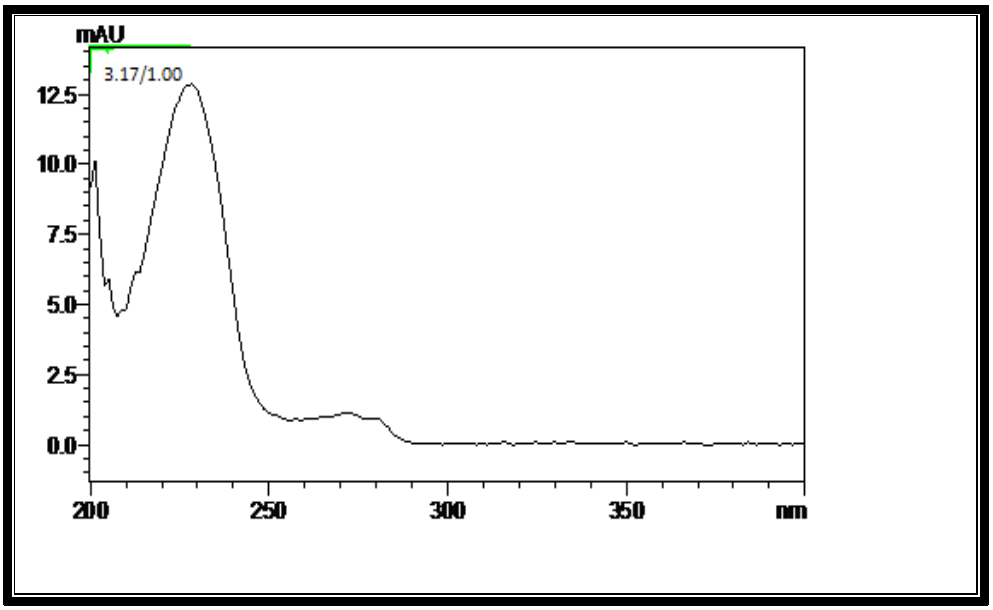


Fig. 39.Chromatogram of sodium benzoate extracted from MAA(2)

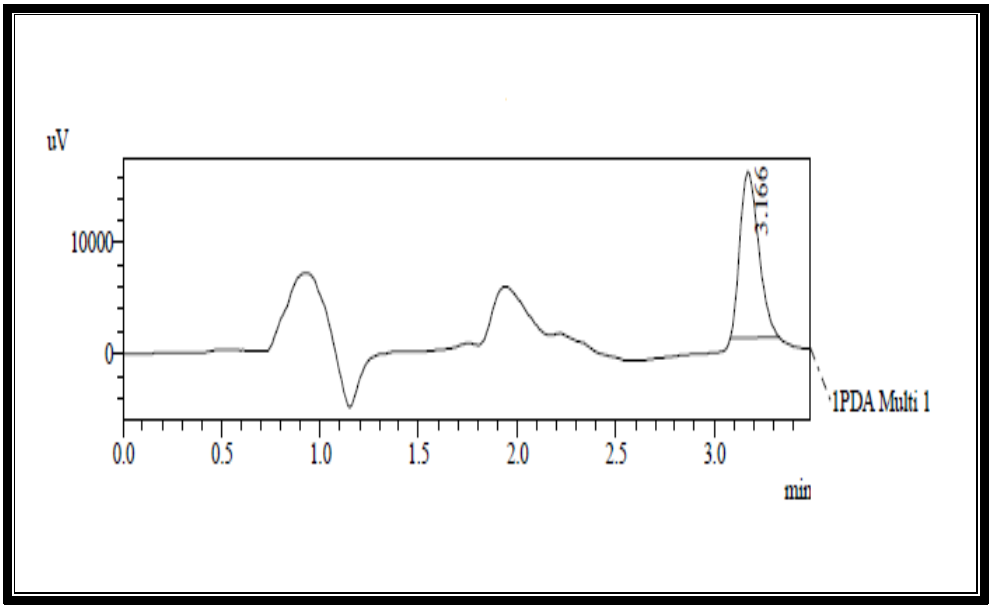


Fig. 40. Spectrum of sodium benzoate extracted from Maa(2)

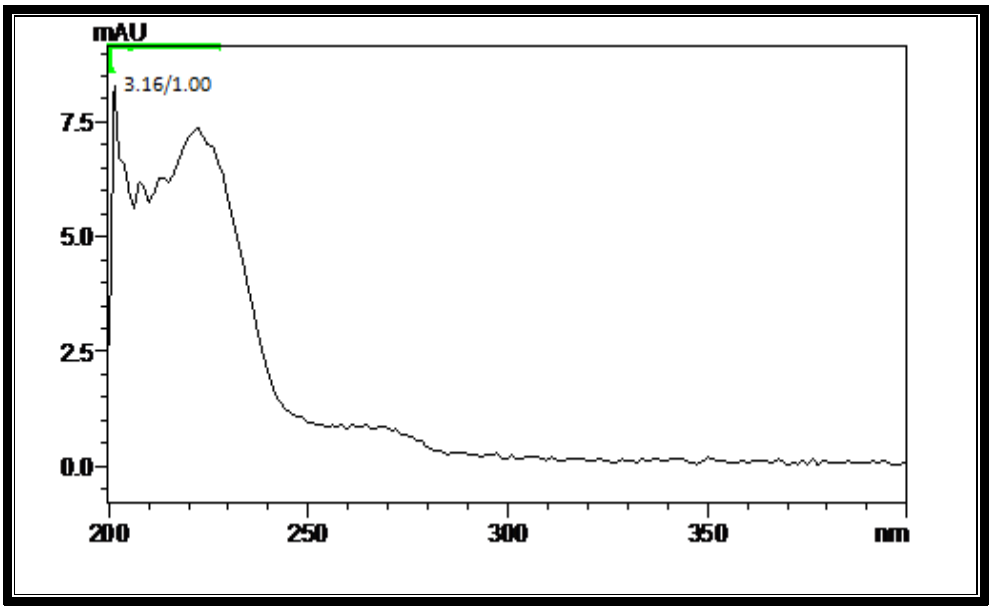


Fig.41. Chromatogram of sodium benzoate extracted from Maa (3)

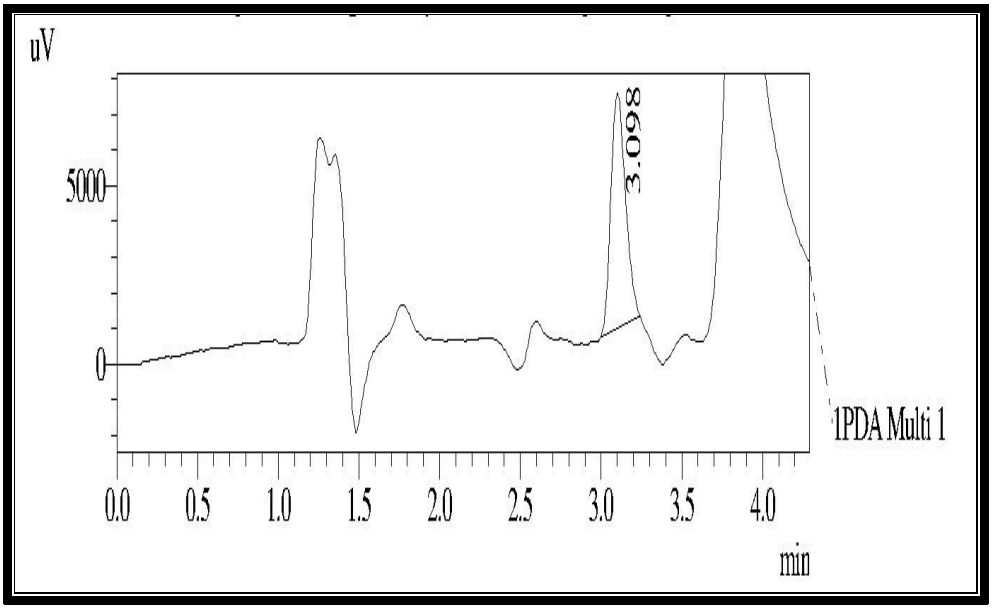


Fig 42. Spectrum of sodium benzoate extracted from Maa (3)

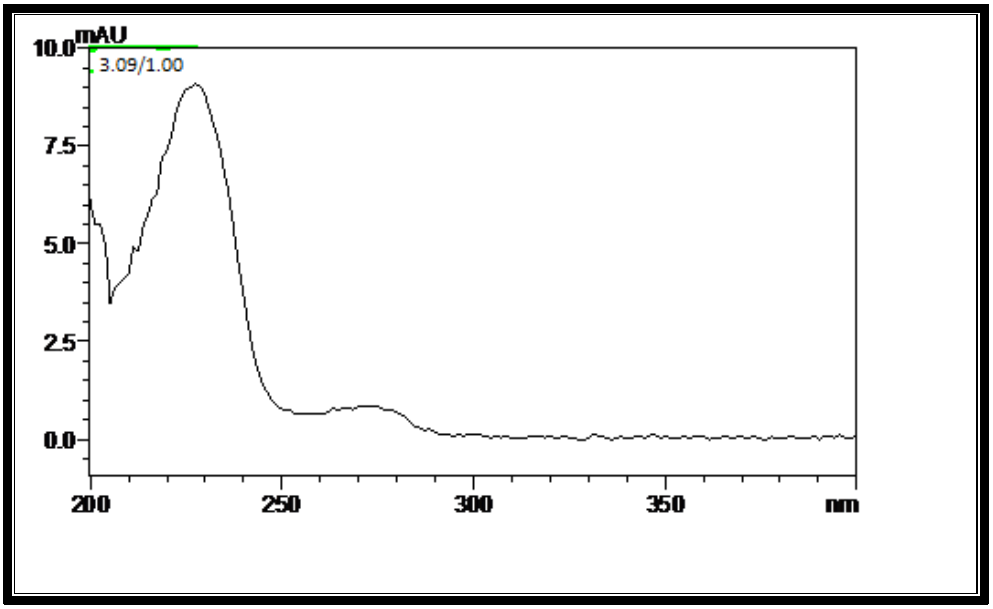


Table 13. Results of analysis of Maa for sodium benzoate

S.No	Product	Peak area	Concentration (µg/4ml)	Present in 600ml(µg)
1.	MAA-1	71227	1.42	213
		72917	1.44	216
		69263	1.39	208.5
2.	MAA-2	64297	1.33	199.5
		63310	1.32	198
		59297	1.27	190.5
3.	MAA-3	59897	1.28	192
		60291	1.28	192
		61217	1.29	193.5
			Avg	200.3µg

RECOVERY STUDIES

To prove the accuracy of the method recovery study was carried out by standard addition technique at 100% level. The standard sodium benzoate was added to each type of the sample and reanalysed by proposed method. The percentage recovery was calculated using formula and shown in table 14.

Table 14.Results of Recovery study

Sample	Amount added.	Percentage recovery
Mirinda	1.5mcg/10ml	99.52
7up	1.5mcg/10ml	100.15
Hydrotonic drink	1.5mcg/10ml	98.72
Frooti	1.5mcg/10ml	99.52
Maa	1.5mcg/10ml	99.17

SUMMARY AND CONCLUSION

Preservatives are essential food additives added to all kind of ready to eat / drink foods. Sodium benzoate is one of such preservative used in soft drinks and fruit juices.

A newer high performance liquid chromatographic method was developed for estimation of sodium benzoate from selected food products like soft drinks and fruit juices. The chromatographic separation was performed on stationary phase of Lichrospher ® 100 RP-18e (5µm) and mobile phase of 20mM ammonium acetate (pH 3.5) : Methanol and Solvent ratio of 25:75, % v/v, flow rate :1.0ml/min. The detection wavelength was at 232nm.

The method was validated for linearity, precision, stability and robustness. The linear concentration was 2-10 mcg/ml and the correlation value was 0.996. The percentage RSD values of repeatability, inter day and intra day precision were found to be <2. It proves precision of the method. The plates were stable upto 48 hrs at room temperature. The method was robust for very minor change in the changed chromatographic parameters. The method was then applied to estimation of sodium benzoate in soft drinks and fruit juices.

Three soft drinks and two fruit juices were extracted and the extraction was carried out on three products same batch. The amount of sodium benzoate present in both soft drinks and fruit juices was calculated.

The Permitted Level of sodium benzoate were 160mg/ml according to the literature. Analysis of all these soft drinks and fruit juices by the developed HPLC method shows the amount of sodium benzoate in the sample were within the limit. As the developed HPLC method involves simple extraction procedure when compared to reported method they can be effectively employed for estimation of sodium benzoate from soft drinks and fruit juices.

BIBLIOGRAPHY

1. Yuri K, Rosario L. HPLC for pharmaceutical scientists: New York; John Wiley & Sons, Inc; 2011.p. 3-10.
2. Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. 2nd ed. New York: John Wiley & Sons, Inc; 1997. p.1-15,205-213, 234-266.
3. ICH harmonized tripartite guidelines. Stability testing of new drugs substance and products Q2(R_1) 2005 Nov. Geneva, Switzerland .
4. Velsicol Chemical Corporation, Product Information Bulletin: Sodium Benzoate Food Grade, FC http://www.velsicol.com/asps/documents/pibs/ pdfpib42.pdf.
5. World Health Organization. Concise International Chemical Assessment Document No. 6 on Benzoic Acid and Sodium Benzoate; Geneva, Switzerland, 2000; http://www.inchem.org/documents/cicads/ cicad26.htm
6. National Soft Drink Association. About Soft Drinks; updated 10/3/03; http://www.ndsa.org/softdrinks/ History/whatsin.html
7. U.S. Food and Drug Administration. Code of Federal Regulations; Title 21 Volume 6, Section 582.3733 sodium benzoate; U.S. Government Printing Office, Washington, DC, Revised April 1, 2003.
8. European Parliament and Council Directive No. 95/2/EC. Food Additives Other Than Colours and Sweeteners; February, 1995.
9. Pezza, L.; Santini, A. O.; Pezza, H. R.; Melios, C. B.; Ferreira, V. J. F.; Nasser, A. L. M. Anal. Chim. Acta2001, 433, 281–288.
10. Manual of method of analysis food safety and standards authority of India ministry of health and family welfare government of India New Delhi.
11. Importance of analytical instruments in food analysis-www.fndnews.com/article/detnews.asp…..18oct/2006.

12. Clague, J.A & Fellers, C.R.(1934).” Relation of benzoic acid content and other constituents of cranberries to keeping quality”. Plant Physiology (9): 631-636.
13. Australian Federation of consumer Organisation (2004). Official Publication.pg262
14. Piper PW Yeast superoxide dimutase mutants reveal a pro oxidant action of weak organic acid food preservatives Free Radic Biol Med1999 Dec; 27(11-12):1219-27
15. FDA, 2006.”Data on benzene in soft drinks and other beverages,”United states Food and Drug Administration. Accessed June2nd athttp://www.cfsan.fda.gov/~dms/benzene.html
16. S. Antakli , A. Alahmad. Simultaneous Determination of Sodium Benzoate and Potassium Sorbate Preservatives in Foodstuffs Using High Performance Liquid Chromatography. Asian J. Chem .2010 ;22(4): pg.3275-3282.
17. Khosrokhavar R, Sadeghzadeh N. Simultaneous Determination of Preservatives (Sodium Benzoate and Potassium Sorbate) in Soft Drinks and Herbal Extracts Using High-Performance Liquid Chromatography (HPLC) . J. Med. Plants .2010; 9(35).
18. Ahmet Kucukcetn. Determination of sodium benzoate, potassium sorbate, nitrate and nitrite in some commercial diary product research article (arasturmamakalesi) GIDA. (2008); 33(4): pg.159-164.
19. Kj. Tidke and Pr. Solanki. Estimation of preservative from local market beverages of Amravati (M.S.) by HPLC technique. IJRPC.2014; 4(4) : pg.1018-1022.
20. Vilas Khade and Sunil mirgane. High performance liquid chromatography method for the analysis of sodium benzoate. J. sci. eng. Res .2014; 5(10): pg.1382 .

21. Zahra Esfandiari, Mohammad Badiey .Simultaneous determination of sodium benzoate, potassium sorbate and natamycin in Iranian yoghurt drink (doogh) and the associated risk of their intake through doogh consumption. Iranian J publhealth. 2013 Aug; vol-42 (8):pg.915-920.

22. Pylypiw HW JR and Grether MT. Rapid high-performance liquid chromatography method for the analysis of sodium benzoate and potassium sorbate in foods. J.chromatography A. 2000 Jun 23; 883(1-2): pg.299-304.

23. Goksel Altiokka, Bulent Ergun .Validated reverse phase HPLC method for the analysis of the food additive, sodium benzoate, in soft drinks and jams in J Liq Chromatogr Relat Technol .2007;30(8): pg.1125-1136.

24. Bahruddin Saad, Md. Fazlul Baria Bahruddin Saad.Simultaneous determination of preservatives (benzoic acid, sorbic acid, methylparaben and propylparaben) in foodstuffs using high-performance liquid chromatography. Journal of Chromatography A; 2005;1073(1-2): pg.393-7.

25. Burge Asci, Sule Dinc Zor. Development and Validation of HPLC Method for the Simultaneous Determination of Five Food Additives and Caffeine in Soft Drinks. Int J Anal Chem .2015;1(5): pg.1-8.